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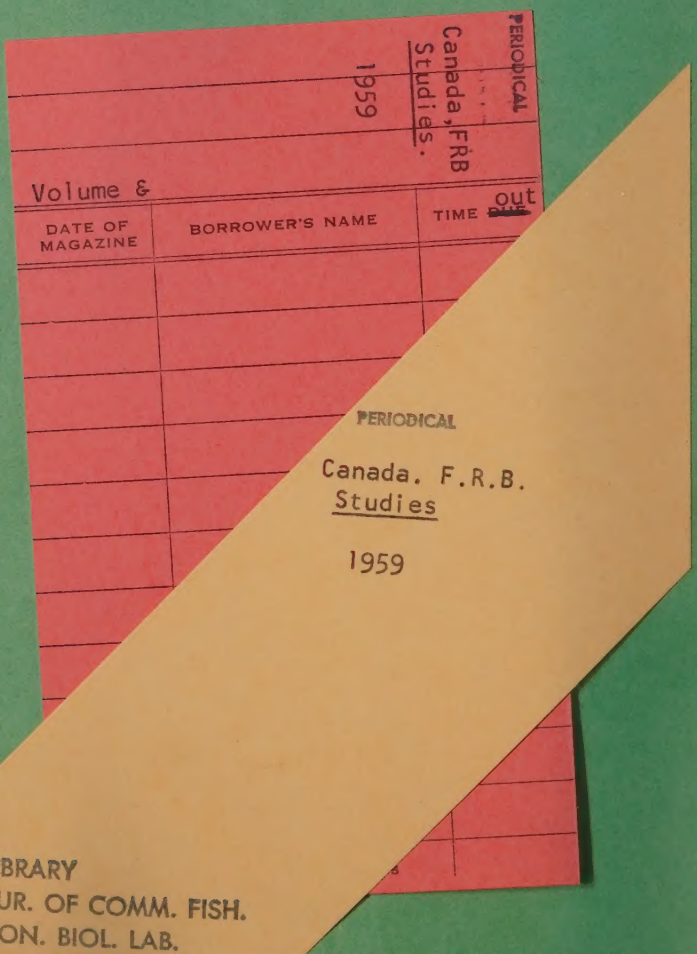
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STUDIES

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RECORDS OF CYAMUS BALAENOPTERAE BARNARD AND NEOCYAMUS PHYSETERIS (POUCHET), TWO SPECIES OF WHALE-LICE (AMPHIPODA), FROM THE NORTHEAST PACIFIC[1]

L. MARGOLIS

Abstract

Cyamus balaenopterae Barnard from *Balaenoptera acutorostrata* and *Neocyamus physeteris* (Pouchet) from *Physeter macrocephalus* are reported for the first time from the Pacific Ocean. This is the first record of a cyamid from *B. acutorostrata*.

Cyamus balaenopterae Barnard, 1931 and *Neocyamus physeteris* (Pouchet, 1888) Margolis, 1955, collected from whales taken in the Northeast Pacific off the coast of British Columbia, have recently been identified by the author. These records represent the first of these two species in the Pacific Ocean. They were previously known from the Atlantic Ocean and they probably occur in the Antarctic. The distribution of *C. balaenopterae* and *N. physeteris*, like other cyamid species, probably parallels that of their hosts.

Cyamus balaenopterae Barnard

Nine specimens (five males and four females), which proved to be *C. balaenopterae*, were given to the author by Dr. C. G. Carl of the Provincial Museum, Victoria, British Columbia. The specimens were collected by Mr. Patrick M. Martin from a *Balaenoptera davidsoni* (= *B. acutorostrata*), the minke whale, little piked whale, or lesser rorqual, taken in Goletas Channel, British Columbia, on August 2, 1940. This is the first published account of cyamids occurring on *B. acutorostrata*.

The males measure 3.7 to 5.0 mm in total body length and the females measure 3.1 to 4.2 mm in length. Barnard (1, 2), who described this species from *Balaenoptera physalus*, the fin whale, and *Balaenoptera musculus*, the blue whale, from South Africa, gave the maximum length of males and females as 8.5 mm and 8 mm, respectively. The females in the present collection are obviously immature because the oostegites are far from completely developed to form the incubatory pouch. Because of the small size, the males probably also are immature. In spite of their immaturity, the specimens from *B. acutorostrata* exhibit all the distinguishing characteristics of *C. balaenopterae* as described by Barnard. I have also had the opportunity to confirm their identity by direct comparison with some of Barnard's specimens which were kindly loaned to me by Dr. Isabella Gordon of the British Museum (Natural History), where these specimens are presently housed. The presence of a prominent single accessory gill at the base of each gill in males, a readily observable feature even in the small males examined by the author, offers a good diagnostic character of *C. balaenopterae*. Other species of *Cyamus* possessing single, elongate, cylindrical gills on each side of peraeon segments

[1]Manuscript received August 7, 1959.
Contribution from the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

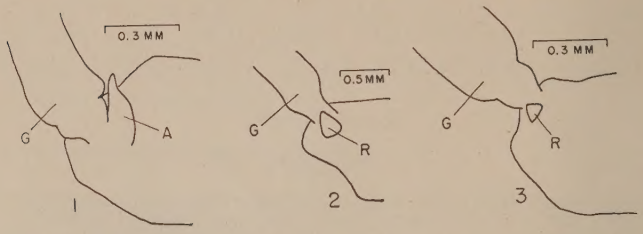

FIG. 1. *Cyamus balaenopterae*. Ventral view of gill and accessory gill of right side, segment 3, of male, 4.9 mm length.
FIG. 2. *Cyamus boopis*. Same, segment 4, of male, 6.5 mm length.
FIG. 3. *C. boopis*. Same, segment 3, of male, 4.6 mm length.

All figures drawn with the aid of a camera lucida. A = accessory gill. R = rudimentary accessory gill. G = gill.

3 and 4, except *Cyamus nodosus* Lütken, have biramous accessory gills. Single accessory gills (although less developed than those in *C. balaenopterae*) also are present in *C. nodosus*, but this species is unique in the possession of longitudinal grooves on the dorsal surface of peraeon segments 3 to 7.

The single accessory gills of males of *C. balaenopterae* (Fig. 1) are unlike the single spine-like rudimentary accessory gills of juveniles of species whose adult males possess biramous accessory gills. For example, in juvenile males of *Cyamus boopis* Lütken (Figs. 2 and 3) of lengths up to about 7 mm the rudimentary gills are, in outline, roughly in the form of an equilateral triangle, have their apices directed towards the mid-line of the gills or somewhat posterior thereto, and do not extend beyond the lateral margins of the peraeon segments; whereas in *C. balaenopterae* males the accessory gills are elongate, with their apices directed anterolateral to the gills, and extend beyond the margins of the peraeon segments (at least in specimens of 4.2 mm or more in length).

Barnard's (1, 2) accounts of *C. balaenopterae* are the only published records of this species. Ohno and Fujino (7), and Kakuwa, Kawakami, and Iguchi (4) reported unidentified cyamids from *B. physalus* and *B. musculus* captured in the Antarctic from the 1946–47 to 1951–52 whaling seasons. For the 6-year period the mean incidence of cyamid infection of 7794 *B. physalus* and 3343 *B. musculus* was about 4.8% and 4%, respectively. In view of the host specificity generally exhibited by cyamids it is likely that the Antarctic specimens were *C. balaenopterae*.

Mr. G. C. Pike, biologist-in-charge of marine mammal investigations for the Fisheries Research Board Biological Station at Nanaimo, has informed me that he has observed cyamids on a small number of *B. physalus* taken by the commercial whaling fleet operating off the coast of British Columbia. Specimens have not been collected but again the species is likely to have been *C. balaenopterae*.

Because of the general lack of opportunity for transfer of cyamids from one whale species to another, I think that the occurrence of *C. balaenopterae* on three of the five species of *Balaenoptera* indicates that this cyamid was present on the common ancestor of *Balaenoptera* spp., and that speciation of the hosts has progressed without simultaneous speciation of their cyamid

parasites. Further field collections may also reveal the presence of this cyamid on *Balaenoptera borealis*, the sei whale, and *Balaenoptera brydei*, Bryde's whale.

Neocyamus physeteris (Pouchet, 1888)

Two males and one mature female of *N. physeteris* were collected by Mr. Dale W. Rice, visiting biologist from the United States Fish and Wildlife Service, on April 7, 1959, from a female sperm whale (*Physeter macrocephalus*), length 34.5 ft, landed at the commercial whaling station at Coal Harbour, British Columbia.

N. physeteris possesses many characters which distinguish it from other cyamids, amongst which the fasciculate gills are the most striking.

This species is the second cyamid to be recorded from *P. macrocephalus* (= *catodontis*) in the North Pacific. Margolis (5, 6) previously described *Cyamus catodontis* (= *C. boopis* var. *physeteris* Pouchet, 1892) from this whale. Both species have been known for many years from sperm whales from the Atlantic Ocean. Clarke (3) recently briefly reviewed the published records of cyamids on sperm whales and noted the lack of records of *N. physeteris* from the Pacific Ocean.

The history and synonymy of *N. physeteris* have been reviewed by Margolis (6), who named the genus *Neocyamus* for *Cyamus physeteris* Pouchet. The Atlantic localities from which this species definitely has been identified are the Azores and Bermuda (Pouchet (8, 9), Verrill (10)). Ohno and Fujino (7) and Kakuwa, Kawakami, and Iguchi (4) have recorded unidentified cyamids from 303 (about 20%) of 1542 sperm whales taken in the Antarctic during the whaling seasons of 1949–50 to 1951–52. In view of the occurrence of *N. physeteris* in the Atlantic Ocean and Pacific Ocean, indicating a wide distribution of this species, some of the sperm whale cyamids from the Antarctic were probably *N. physeteris*.

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INSECTICIDES AND WILDLIFE

By M.H.A. Keenleyside
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These young Atlantic salmon were found dead along the banks of a New Brunswick stream a few days after DDT was sprayed on the surrounding forest.

IN 1957 over 5,000,000 acres of northern New Brunswick wood land was sprayed with DDT in the continuing fight against the spruce budworm epidemic which is raging through the province's balsam fir and spruce forests. The insecticide was dissolved in a special oil and sprayed from aeroplanes at a rate of one-half pound DDT per acre. The entire job was done in about four weeks between early June and early July by a fleet of 200 aeroplanes operating from 14 airstrips built at various points in the bush. Large-scale insecticide spraying of this sort has been done in New Brunswick each year since 1952, although the 5,000,000 acres sprayed in 1957 was the biggest job done in any one year. Spraying began in 1952 when it was decided that the budworms were so thick in parts of northern New Brunswick that only immediate spraying with insecticide could save many of the trees from death. Since that year the area of very heavy infestation has spread in a southerly and easterly direction. By now some regions have received 2, 3 or even 4 doses of DDT, as the budworms have repeatedly moved back in and reinfested sprayed areas.

Soon after the huge 1957 spraying job began, dead and dying fish were found along the banks of some rivers flowing through the sprayed woods. Among several species found dead were young Atlantic salmon and brook trout, both highly prized sport fish. There were also reports of dead birds being found in the woods and motorists reported seeing flickers and several kinds of small songbirds dead along roads in the sprayed areas. There were even rumours that deer had been found

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PHOTO BY ONTARIO DEPT. OF LANDS AND FORESTS

Close-up of spruce budworm. The insect attacks the tree from the top and this has a tendency to rot and fall off.

Dr. M. H. A. Keenleyside, the author, was born and educated in Ottawa. He graduated from the University of British Columbia in Dept. of Zoology, and received his Ph.D. degree under Dr. G. P. Baerends, University of Groningen, Holland. Since 1955 he has been working for the Fisheries Research Board of Canada, at present associate scientist in the salmon investigation at St. Andrews, N.B.

dead in sprayed woods with no signs of external injury.

These stories and rumours soon led to a typical argument. On one side were the spraying authorities and many people whose living depends on the pulp and paper and the lumbering industries. They said there was little if any actual proof that all those fish and birds had died as a result of the DDT spray. Lately there have been more reports than usual about the harmful effects of DDT on wildlife, they said, and people are more likely to be looking for dead animals in the woods. Birds which may have died of natural causes and which went unnoticed before, were now found by anxious sportsmen. The dead deer story was never proven, they pointed out. Some, in fact, refused to admit that DDT has killed any fish or wildlife in the province, or if it has, that the numbers involved are so small compared to the countless thousands surviving that it made no difference.

On the other side of the argument was a growing body of sportsmen, naturalists, and biologists. Many of them are fearful of the consequences of blanket-type spraying of insecticides over large areas, with its accompanying upset of the "balance of nature." This group is becoming increasingly worried, as there seems to be no end to the use of poisons, in ever increasing amounts, to control more and more pests. Some argue that *any* damage to wildlife is a bad thing and when vast areas of woodland are sprayed each year, as in northern New Brunswick, there is bound to be damage to the birds, mammals and fish living in these woods.

This kind of argument is as old as the history of chemical control of pests. There have always been those who were concerned over the use of chemicals to kill undesirable animals. Their concern mounted after the second world war with the increasing use of DDT, and of the much more toxic substances discovered later. The relative effectiveness and low cost of DDT led to the beginning of large-scale, blanket spraying from aeroplanes, of thousands of acres in a short period. The opponents of spraying argued that under such

PHOTO BY THE ROYAL ONTARIO MUSEUM

The Atlantic salmon that is radically affected by the use of insecticides.

Young salmon, sticklebacks and aquatic insect larvae killed by DDT that entered the stream in which they lived.

conditions many desirable and harmless animals cannot escape contact with the insecticides, either directly or through eating poisoned insects. At the same time, there have always been those who played down or ignored the possibility of dangerous side-effects, provided that good control of the pest was achieved.

The danger in this sort of quarrel lies with the extremists on both sides; those who have closed minds on the subject, who discourage impartial investigation, and who slant all reports to suit their prejudices; those who become emotional, irrational, even hysterical when discussing insecticides versus wildlife. What is needed is reasonable, unbiased judgment of each case, based on the facts. And simply because the facts are so often difficult to find, irrational arguments have been all too common.

This does not mean that no attempts have been made to assess the effects of insecticides on wildlife, either in the laboratory or under actual operating conditions in the field. A recent publication by two University of California zoologists reviewing the relations between pesticides and wildlife has a list of over 1,000 published papers on the subject. Almost all of these were written since 1945, reflecting the recent and rapid increase in the use of chemicals to control pests.

This great volume of work has been both reassuring and frightening. Many investigators found no evidence that spraying of insecticides over forests or agricultural crops was harmful to the fish or wildlife living there. In some cases a few deaths were recorded, but were not considered important. However, few of these studies were long-term. Usually there was little or no information on the numbers of animals in an area before spraying for comparison with the numbers found later. Also, few of the investigations were carried through to see if undesirable effects were passed on to later generations. On the other hand there are a great many proven cases of fish, bird, and mammal mortalities following insecticide

Photos by the FISHERIES RESEARCH BOARD OF CANADA unless otherwise credited.

Fish census on the Northwest Miramichi River, N.B. The man on the extreme left is operating the electro-fishing gear (the positive electrode is in his right hand). Another man picks up stunned fish with a dip net while two others hold a seine to catch fish which escape the dip net.

spraying for the control of insects. Some animals have been killed by direct contact with the poison; others have died after eating insects killed by the spray. In general, the stronger the insecticide, or the higher the concentration at which it is applied, the greater the number of deaths occurring among the wildlife of the treated area.

Some of the most spectacular mortalities have been due to accidents, to spraying techniques which do not take into account the habits of the wildlife in the area, or to insufficient control over the amount of poison in the spray. For example, in 1947 an insecticide containing the extremely toxic compound TEPP was sprayed onto orchard trees in Washington state. The job was done at night because the spray remained toxic longer at the cooler night temperatures. Pheasants roosting in the trees were not flushed by the spraying operation and large numbers were killed where they roosted. The fact that too much TEPP was in the spray also contributed to many of the bird deaths. Since 1948 the use of TEPP has been more closely controlled and wildlife losses greatly reduced.

One of the best established cases of wildlife mortality connected with the use of insecticides is in New Brunswick. My story began with a description of the 1957 spraying against the spruce budworm in New Brunswick and the subsequent argument typical of many arising after large-scale operations of this kind. At that time a systematic scientific research program was going on to determine the effects of DDT on the fishes of the province, and particularly the salmon. This program has been carried on for several years by the Fisheries Research Board of Canada, from its Biological Station at St. Andrews, N.B. It has found good evidence that control of spruce budworms by aerial spraying of DDT has had serious effects on the salmon of several New Brunswick rivers. Since it is unique in scope and duration, this program is worth describing in some detail.

Beginning in 1950 an annual census has been taken of the fish

Caddisflies, mayflies, blackflies, and other insects are removed daily from this type of cage which covers one square yard of stream bottom.

living in the Northwest branch of the Miramichi River, one of the province's best salmon rivers. Since salmon in this part of New Brunswick usually spend three years in fresh water before going out to sea, there are salmon of three different ages in the river at the time of censusing. In addition, brook trout, suckers, eels, and several species of minnows are taken in the census. Fishing is done by a well trained crew using nets and electro-fishing equipment. Sample areas at intervals along the river are blocked off by barrier nets set across the stream so that no fish can get in or out during censusing. The fishing records are collected and treated statistically by Dr. P. F. Elson of the St. Andrews Biological Station, who is able to make an accurate estimate of the numbers of salmon and other fish in each sample stretch of the river. He then determines average populations for the whole river. The census has been taken in this way each year since 1950 and the results have clearly shown that DDT spraying is harmful to fish, and especially to the young salmon.

DDT spraying against the spruce budworm began in 1952 in a small section of northwestern New Brunswick and has been carried on annually since then. In 1954 the watershed of the Northwest Miramichi River was sprayed for the first time. During the fish census of that year, done about two months after the spraying, no underyearling salmon (those hatched in the spring of the same year; often called fry) were found at any of the ten census stations. Some 1- and 2-year-old salmon were caught, but they were much scarcer than in previous years. In addition, many yearling salmon held in cages in streams inside the sprayed area died shortly after spraying, while fish held the same way in streams outside the sprayed woods survived well.

It appears that DDT may be blown onto waterways by the wind after it is released from aeroplanes, it may be washed into the rivers by heavy rains soon after spraying, and, in some cases, pilots of spray planes cannot see small headwater streams and spray right over them. In any case, it does not have to be in the water in high concentrations to be dangerous to fish. Laboratory studies have shown that very small amounts of DDT in water are sufficient to kill young Atlantic salmon and also that, in general, smaller fish are killed at lower concentrations than larger fish.

During the past few years the annual fish censusing has been extended to several other salmon streams in New Brunswick. Wherever a census has been taken on rivers flowing through recently sprayed forest the picture has been much the same as on the Northwest Miramichi. Young salmon of all sizes are scarcer than usual; the youngest are often practically wiped out. In one branch of the Miramichi, the Cains River, whose watershed has not yet been sprayed with DDT, young salmon are as abundant as usual.

The effect of these deaths on the numbers of adult salmon available for fishermen a few years later is likely to be serious. An article in a recent issue of the Atlantic Advocate based on the calculations of Drs. C. J. Kerswill and P. F. Elson of the St. Andrews Station predicts a drop in the salmon catches of several famous New Brunswick rivers over the next few years.

In addition to the fish mortalities many of the aquatic insects on which the young salmon feed are also killed by DDT as a result of the budworm spraying.

The larval stages of many caddisflies, stoneflies, mayflies, etc., live among the stones on the bottom of fast flowing, cool streams, just the areas where young salmon thrive. Careful studies of the adult insects produced in several areas of the Northwest Miramichi River since 1954 have shown clearly that DDT, sprayed on the surrounding watershed, greatly reduces the numbers of stream insects. Some types, including the bigger caddisflies which are choice food for the larger young salmon, are very slow to return to the areas where they have been killed off. This means that the young salmon which survive the DDT may have a hard time finding enough food for proper growth.

To try and speed up the return of these valuable fish-food organisms to sprayed areas, an attempt was made in 1957 to transplant aquatic insects from unsprayed streams where they are still abundant. At the same time, hatchery-reared salmon are being introduced to streams where many wild fish have been killed by DDT. However, one of the disheartening aspects of the New Brunswick budworm outbreak is that it is so severe and widespread that most infested areas now have to be resprayed after intervals of one or two years. This likely means that the repopulation measures will not succeed, because the introduced fish and insects themselves may soon be poisoned by DDT.

Recent publicity over the bad effects of budworm control on the salmon of New Brunswick has again raised the old argument over chemical pest control. Loud and sometimes emotional voices are again being heard on both sides and the argument is not confined to the one province. Recently, concern has been expressed over the possible dangers to humans, as well as to wildlife, following a large-scale aerial DDT spray program in 1957 to control a gypsy moth outbreak in New York state. Conservationists there are alarmed over the possibility of ever-increasing amounts of DDT finding their way into fruit, vegetables and even milk. This case recently reached the law courts, where a strong attempt was made to prevent further spraying of this kind in the state.

Support is increasing for the prevention of all large-scale applications of insecticides where there is any danger to wildlife. Some of this support is due to increasing public awareness of the mortalities that have been caused by blanket spraying. Also, the world-wide publicity now being given to radioactive fallout from nuclear explosions has awakened many people to the possible dangers of contaminating large areas by any artificial means, including aerial control of insects.

At the same time there is stronger support than ever for those favouring chemical control. In New Brunswick, for example,

NOTE:

The massive broadcasting of pesticides is of growing concern to conservationists. Chemical poisons are mounting in distribution, variety and toxicity almost daily. Bewildering arrays of such preparations are being frantically used as emergency stop-gaps against "pest" infestations—without adequate prior knowledge of their possible cumulative effects on wildlife and human life.

Two Stearman biplanes spraying a DDT-in-oil solution on spruce budworm infested trees in New Brunswick.

The Audubon Society of Canada believes that there is an onus on the users of pesticides to prove the safety of toxic chemicals before they are put to use. Careful research is needed without delay. What little research has been done still lags far behind the actual practice of poisoning, but it has already indicated that the dangers are immediate and extreme.

Nature's balance is a delicate and precarious one. We cannot interfere with it lightly. It is quite possible that the indiscriminate spraying of chemical poisons may represent the greatest potential threat to life of all kinds that the world has yet experienced.

The Editors.

many more people depend on the forests now than during the last serious budworm outbreak, which occurred about 1915. At that time there were no chemical control methods and the outbreak was allowed to run its course. In the process many trees died. Now, because the stakes are larger, the pulp and paper companies do not want to let their trees die. They are willing, even anxious, to save the trees by chemical control, even at the cost of millions of dollars each year. They agree that at the beginning of this outbreak it was thought a few years of spraying would control it. Although these early expectations were obviously too optimistic, the woods operators justify the use of insecticides in terms of numbers of trees they believe have been saved from death.

It is quite possible that a period of reconciliation is approaching. Each side in this argument must recognize the interest of the other in the woods. Also, some facts must be faced squarely and honestly. Chemical control of some of the insect pests is inevitable and, on the whole, probably desirable, if the majority of the people of the country stand to benefit from its use. Highly poisonous chemical materials are here to stay and we must learn to live with them. After all, one of the chief signs of advancing human civilization is our greater ability to change or redirect the ways in which animal and plant communities develop, either in cooperation with, or in spite of the environment. The proper use of chemicals to control what we believe are the harmful insects when they reach epidemic numbers is only one aspect of this ability. We should remember that one reason for sudden increases in forest insect populations is the method we have used to harvest the trees in the past. We ourselves have made the forests unnatural by our cutting methods and by inadequate reforestation, and when these factors contribute to pest outbreaks it is illogical to shrink, on principle, from using artificial methods to control the pests.

On the other hand, we must keep ourselves well informed of the risks involved, particularly as the newer insecticides are so very toxic and since we have the means for distributing them rapidly over very large areas. Studies into the effects of widespread spraying on our wildlife resources must be scientifically sound and must be long-term. Much more research is needed into the problems of how animals come in contact with poisons, how much they can tolerate without harm to themselves or their offspring, what restrictions on the methods of applying the insecticides are feasible, etc.

Obviously we cannot allow valuable local plant resources to be wiped out by uncontrolled insect attack. Not only would there be serious economic and social consequences but our wildlife depends in the long run on these plant resources as well. Neither can we allow ourselves to consider our wildlife as expendable. We need our wildlife, and we will need it more in the future. With shorter working hours and higher incomes that go with an increasing standard of living many more of us are taking to the outdoors for relief from the stresses and strains of modern life. And although many want to hunt and fish, others get their relaxation by simply getting outdoors and observing our native plants and animals. We must not belittle the recreational and aesthetic values of our wildlife, even though it is almost impossible to put accurate price tags on these values for comparison with industries that have an economic stake in the same areas.

Finally, we should not get into the habit of using insecticides as the easy way out. When insect outbreaks occur, all possible alternatives must be considered. Biological or natural controls (such as predators, parasites or viruses) should be tried. The search for more specific chemicals that will kill the pest species, but not harm other animals, must be continued. When spraying must be done, the greatest possible care should be taken to prevent accidental mortalities. And perhaps most important of all, we must improve our methods of managing agricultural and forest crops and thus reduce the chances of pest populations building up to epidemic size.

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PART 4

IV. Observations on the Cod Trawl Fishery in the Gulf of St. Lawrence During the Spring of 1958.

BY

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AND

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At the 7th Annual Meeting of ICNAF the French delegation invited Canada and the United States to send biologists to observe fishing operations of trawlers in the 1958 spring fishing season, and to sample their catches. The appropriate arrangements were made through the Federation des Syndicats d'Armateurs à la Grande Pêche, and the trawler "Zelande" picked the two writers up at Sydney on March 15 and proceeded to Subdivision 4R where fishing commenced on March 16. McCracken transferred to the "Clairvoyant" on March 17th and remained there until March 25. Clark remained aboard the "Zelande" until March 21, went aboard the Portuguese trawler "Santo André" for five days and returned to the "Zelande" from March 26 to March 30.

Thanks are particularly due to M. Dezeustre of Pêcheries Bordeaux, Bassens, Bordeaux, and M. Girard of Les Chalutiers Malouins, Saint Malo, for their arrangements, as well as to Captain LeCoeur of "Clairvoyant", Captain Thomas of "Zelande" and Captain São Marcos of "Santo André" for their co-operation and assistance.

Areas fished.

The trawlers fished in depths of 80-300 (mostly 200) m off the west coast of Newfoundland (4R), in the central Gulf of St. Lawrence, off the northeastern part of Cape Breton Island (4T),

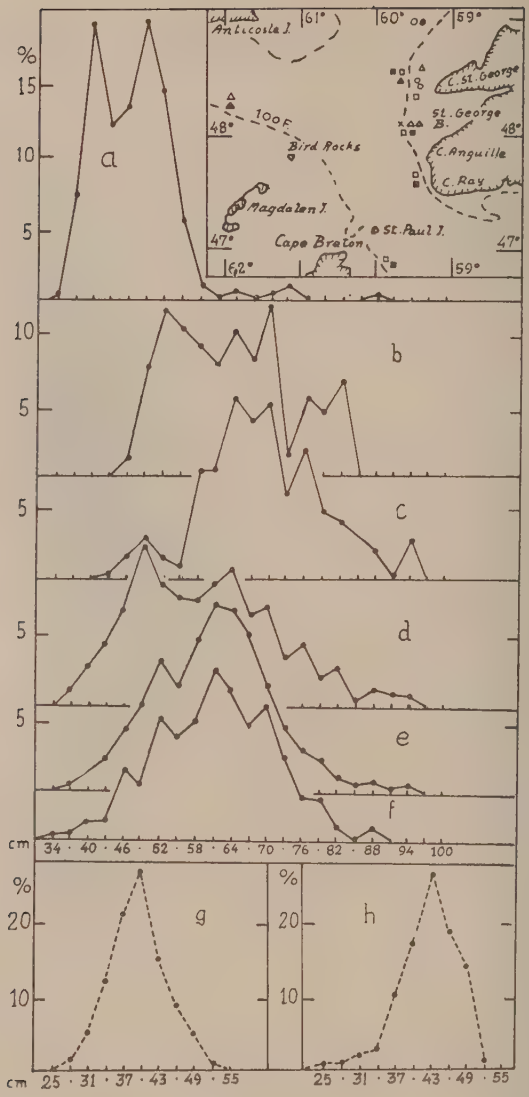

Fig. 1. a—f: Comparative length distribution (3 cm groups) of catches of cod (unsorted and retained samples). g and h: samples of discards. Map showing sampling localities inserted. a—Bird Rock 4T, b—St. Paul I. 4V, c—N. of Cape George 4R, d—W. of Cape George 4R, e—N. of Cape Anguille 4R, f—off Cape Ray 4R, g—W. of Cape George 4R, h—off Cape Ray 4R.

and southeast of St. Paul Island (4V). Localities where catches were sampled are shown in Figure 1, inserted.

Fishing Gear.

The size of gear used by all three trawlers is essentially the same, with headrope about 80 feet, footrope about 110 feet, and about the same mouth opening as those used by Canadian and United States large otter trawlers. The body of the trawl was in all cases of manila. French vessels were making extensive use of nylon in the codend and lengthening piece. The nets had much longer lengthening pieces and codends than North American trawlers. Codend mesh size was 4-4½" for nylon and 5½" for manila.

Catches.

The catches of the three trawlers consisted mainly of cod. In Subdivision 4R catches were usually 70-80 thousand pounds per day, in 4T and 4V much smaller. Catch per tow (2 hours) varied between 1,000 and 70,000 pounds. Some redfish were taken in most hauls. A few catches of redfish of up to 5,000 pounds were seen, off Cape St. George, Cape Anguille and Cape Ray at depths of 80 fathoms and over. Some haddock were taken in most tows in 4R. Only occasionally were they taken in quantity, particularly off Cape Anguille and Cape Ray. Pollock were taken occasionally. Other species noted were halibut, American plaice, wolffish, silver hake, skates, lumpfish, grenadiers and shrimps.

A series of observations on feeding and maturity of the cod were made.

Length of Cod.

Cod measurements were taken in all the major fishing areas visited (the data will be given in tabular form in the "Sampling Yearbook"). Most of the sampling was of that portion of the catch which was retained for salting. In three instances these samples were supplemented by measurements of the discarded portion of the same catch. Two samples were obtained of the unsorted catch and included some discardable sizes. The discard was so light, usually, that

lengths of fish in the sorted and unsorted catches differed little.

The samples were placed into groups representing six fishing areas (Figure 1). The smallest cod were in the Bird Rock Island area (4T), 40-55 cm. In the St. Paul Island area (4V), to the south, the fish were considerably larger, 48-71 cm. In 4R the modes were between 60-65 cm and 48-53 cm. The Cape Ray and Cape Anguille fish were similar in length. Smaller fish were taken west of Cape George and larger fish north of Cape George.

Discarded Fish.

Only small amounts of cod were discarded in the areas fished, the greatest amount in any of the areas visited making up about 10 per cent of the weight of the total catch.

All redfish taken were discarded, but catches did not exceed 5,000 pounds per tow in any area. Only negligible quantities of the smaller sizes of other food fishes were discarded.

From measurements of discarded fish (bottom curves in Fig. 1) it can be concluded that cod of less than 36 cm are not usually retained.

(Reprinted from Annual Proceedings, Vol. 8, International Commission for the Northwest Atlantic Fisheries, pp. 83 to 98, 1958.)

PART 4

III. Summary of Gear Selection Information for the Commission Area

BY

JOHN R. CLARK, FRANK D. McCRACKEN, AND WILFRED TEMPLEMAN

INTRODUCTION

At the Joint Scientific Meeting of ICNAF, ICES (International Council for the Exploration of the Sea), and FAO (Food and Agriculture Organization of the United Nations) at Lisbon in 1957 working groups considered many aspects of our knowledge of gear selection. They reviewed the current status of knowledge and suggested further important research for each of these aspects. At the final session of this Joint Meeting it was concluded that many gear selection problems should be related to specific geographical areas and recommended that the status of gear selection knowledge in the ICNAF area should be examined in order to establish priorities for necessary gear research.

Two of the authors, Clark and McCracken, were asked to carry out this review and to report their results to the Research and Statistics Committee of ICNAF at the Eighth Annual Meeting. Templeman was subsequently asked for his assistance in preparing the redfish review.

We have taken as our basic terms of reference the species caught in the ICNAF area and the gears used to catch them. A limited amount of published data is available, but our sources of information are chiefly the papers presented at the Lisbon meeting (to be published by FAO). We have used primarily the results of gear selection experiments from the ICNAF area, but Northeastern Atlantic results were included where necessary.

We have considered only "inherent" (or intrinsic) selection by gear; that is, selective properties which can be modified by changing certain properties of the gear. We have not considered non-inherent (or extrinsic) selection, which results from such factors as non-representative distribution of fishing effort over the whole range of sizes of a stock of fish.

We are indebted to our colleagues on the Research and Statistics Committee of ICNAF for their helpful comments and suggestions on preparation of the manuscript. We are particularly grateful for the thoughtful and detailed criticisms rendered by Mr. Basil B. Parrish and Dr. Erik M. Poulsen.

SUMMARY OF SELECTION KNOWLEDGE

In order to present the summary of knowledge in the most concise manner possible we have reduced the text to a minimum of essential material. General background material concerning selection processes is included in Appendix I. Detailed derivations of the selection estimates are presented in Appendices II-VI.

Otter Trawls.

An extensive series of experiments with otter trawls has been carried out in the ICNAF area. The majority of the work has been concentrated on haddock, with lesser amounts of work on cod; redfish; American plaice, *Hippoglossoides platessoides* (Fab.); witch, *Glyptocephalus cynoglossus* (L.); and silver hake *Merluccius bilinearis* (Mitch.). Most of these results and results from a considerable amount of European work are available in the Lisbon papers and in other publications.

Escapement through forward parts of the trawl.

Replicate tow experiments with haddock in Subarea 5 have indicated in general that escapement through the lower wings and belly was substantial; but that escapement through top wings, square, and upper belly was relatively unimportant. The total numbers escaping from all forward parts of a trawl are lower, however,

than for a codend of the same mesh size; the estimated 50% retention point for the No. 41 trawl, of $4\frac{3}{4}$ inch mesh (internal size), excluding the codend, occurred 5 to 12 cm lower than for a codend of equivalent mesh size. In addition escapement through the forward parts of the net may be of even lower practical importance, as many of the fish which may be prevented from escaping through forward parts of the trawl will escape through the codend.

Replicate tow experiments with an 84 mm mesh trawl have shown that the forward parts are important for the escapement of silver hake (Clark, 1957c). Most of the fish of less than 16 cm. escaped; about half of the fish of 16-33 cm escaped, with little selective action shown; and most of the fish above 33 cm were retained. No definite 50% escapement length can be chosen because of the extended range over which approximately half of the fish escaped. However, it can be concluded that the forward parts permit substantial escapement of sizes of fish which would have been at least partially retained if they had entered into a codend of the same mesh size. Controlling mesh size in the forward parts of the trawl thus appears more important for silver hake than for haddock.

The data are, however, insufficient to make quantitative estimates of haddock or silver hake escapement for different trawls and mesh sizes. No data are available with which to determine the importance of escapement from the forward parts of trawls for redfish, cod or plaice in the ICNAF area.

Escapement through the codend. In a trawl of uniform mesh size most fish of sizes within the selection range of the codend will escape from the codend rather than through forward parts of the trawl. The precise area of escapement will depend upon the amount of catch accumulating in the codend and the resulting blocking of the meshes. However, for moderate catches the greater escapement occurs in the after 6 or 8 feet. Most codend mesh sizes reported for selection experiments, however, represent averages for the whole codend, rather

TABLE 1. Estimated 50% codend retention lengths for haddock taken by otter trawl. Selection span adjustment = 4 cm.

Mesh Size		50% Retention Length (cm)		
		Double Manila Av. Selection Factor = 3.2	Single Cotton Av. Selection Factor = 3.6	Synthetics Av. Selection Factor = 3.5
mm	in.			
102	4	32	35	35
108	4¼	34	38	37
114	4½	36	41	40
121	4¾	39	44	42
127	5	41	46	44
133	5¼	43	48	46
140	5½	46	52	48

TABLE 2. Estimated 50% codend retention lengths for redfish taken by double manila otter trawl codends. Selection span adjustment = 2 cm.

Mesh Size		50% Retention Length
mm	in.	Av. Selection Factor = 2.5
70	2¾	16
76	3	18
83	3¼	20
89	3½	22
95	3¾	23
102	4	25
108	4¼	27
114	4½	29

TABLE 3. Estimated 50% codend retention lengths for cod taken by otter trawl. Selection span adjustment = 5 cm.

Mesh Size		50% Retention Length (cm)		
mm	in.	Manila Av. Selection Factor = 3.5	Single Cotton Av. Selection Factor = 3.7*	Synthetics Av. Selection Factor = 3.8
102	4	36	38	39
114	4½	40	42	43
127	5	44	47	48
140	5½	49	52	53
162	6	53	—	61

*less reliable data

than the effective escape area only, and these are the figures which we have used.

The estimates of 50% retention lengths for various species in Tables 1 to 5 are based primarily upon experiments in the ICNAF area. The European data have been drawn upon where necessary, such as in the case of the variable haddock results for synthetics.

Estimates are presented for simplicity in the form of 50% retention lengths only. The range

TABLE 4. Estimated 50% codend retention lengths for silver hake taken by otter trawl. Selection span adjustment = 4 cm.

Mesh Size		50% Retention Length (cm)			
		Manila	Cotton	Nylon	
mm	in.	Single or Double	72-84th	400/3 Single	
76	3	21	23	25	
83	3¼	24	26	28	
89	3½	26	29	31	
95	3¾	29	32	34	
102	4	32	35	38	
108	4¼	36	39	41	
114	4½	39	—	—	

over which the selection operates is extremely variable. The reasons for such variations are not well known at present and cannot be discussed in a work of such restricted scope. Average figures have been determined and are provided along with the estimates of 50% retention lengths as selection span adjustments, which may be added to the 50% retention length to obtain the 75% point and subtracted to obtain the 25% point. The total range of codend selection can be taken as roughly twice the 25 to 75% span.

TABLE 5. Estimated 50% codend retention lengths for American plaice and witch taken by otter trawl. Selection span adjustment = 3 cm.

Mesh Size		50% Retention Length (cm)	
		Manila Av. Selection Factor = 2.0	Synthetics Av. Selection Factor = 2.2
mm	in.		
102	4	20	22
114	4½	23	25
127	5	25	28
140	5½	28	31

The derivation of our estimates is detailed in the appendices, where appropriate.

The relation between mesh size and the 50% retention length is linear for all species except silver hake for each type of material. The 50% retention lengths for haddock and redfish have been read from the regression lines shown in Appendices II and III. Because the lines have appreciable intercept values, the selection factor (the ratio of fish length at 50% retention/mesh

size) is not constant but varies with mesh size, but for convenience average selection factors have been estimated for the range of mesh sizes considered and are included with the tables.

Selection experiments for cod only extend over a fairly narrow range of mesh sizes, and in many cases cod have been taken as an incidental species. These data are insufficient to determine whether the selection factor is constant. The 50% retention length estimates in Table 3 are based on lines fitted to the 50% retention length and mesh size data shown in Appendix IV.

The 50% retention length /mesh size relation for silver hake is described better by a curve than a straight line (App. V). The escapement lengths have been estimated from the curve, and as average selection factors are not appropriate for this species, none have been provided.

Results for American plaice and witch suggest that selection factors for these species are similar to those for some of the flatfishes of European waters. The 50% retention lengths for these species, given in Table 5, have been determined in the same manner as for cod.

The Problem of Chafing Gear.

The codend results presented for all species are based upon single-layered codends which are uncovered on their supper surfaces. Various protective devices (chafing gear), such as secondary codends fitted over the primary codend or various covering materials, are commonly used in the ICNAF area. We have a little experimental evidence on the effect of such chafing gear on escapement. Such data as are available show that these protective devices may cause a substantial reduction in fish escapement with certain methods of application.

The considered opinion of ICNAF biologists is that a covering piece of netting should not markedly reduce escapement if affixed loosely and left open at its rearward edge. Whether this opinion is justified and whether chafing gears rigged in other ways could be used without reduction of escapement must await the results of further experiments.

Hook Selection.

Only a limited amount of information is available from which to judge the selective effect of various sizes and shapes of hooks. Little is known about the possible selective effect of various kinds and sizes of bait.

It has been established that hooks fished on longlines select sizes of fish from the stock generally in relation to the size of the hook, with large hooks catching fewer small cod than small hooks. Preliminary attempts to relate hook dimensions to sizes of cod caught appear promising, but as yet there is no well-defined relationship such as the selection factor for otter trawls. From the results available we are able to estimate selectivity values for some hooks currently in use.

Data for handlines are insufficient for reliable estimates but generally indicate that handline selective action may not be so directly related to hook size as that of longlines. The catch of the smaller sizes of cod and haddock, i.e., those within the selection range of 4½ inch mesh codend, is low in the ICNAF area for both methods of hook fishing.

Cod. Some results for hooks fished on longlines are available for the ICNAF area (McCracken 1957a) and off Norway (Saetersdal 1957c). The estimates of selection provided by these experiments are given in Table 6.

TABLE 6. Cod selection estimates for hooks fished on longline.

Hook Type	50% Escapement Length	25-75% Selection Span
No. 17 Mustad	ca. 35-40 cm	?
No. 14 Mustad	48	12-14 cm
No. 7 (Norwegian)	55	14 cm
No. 11 Mustad	65	15-18 cm

For handlines we can only say (1) that results of Saetersdal's experiments suggest similar selectivity for handlines and longlines, and (2) that size composition of cod catches off the Newfoundland east coast suggests that large handline hooks catch the same size cod as smaller longline hooks.

Haddock. Limited results obtained by McCracken (1957a) for longlines provide the only information available for haddock. These results indicate that hooks select sizes of haddock in a manner similar to otter trawls. McCracken's data are too limited to predict a 50% retention length and indicate only that the smallest hook used by Canadian longliners (No. 17) appears to release as many haddock as a $4\frac{1}{2}$ inch mesh codend. Nothing is known about handlines election.

Pair Trawls.

There are no data on selection by pair trawls. They are so different in design and operation from otter trawls that we have not considered inference of their selection properties from those of otter trawls to be valid. Pair trawls used in the ICNAF area are made of natural fibres (manila or sisal). The twine used in these nets is usually light and flexible, the codends are unprotected on top, and their mesh size is larger than that of the otter trawls. Moreover, it is believed that pair trawls fish higher in the water than the conventional otter trawl. Comparable observations on length composition of catches by pair trawls and otter trawls are not available.

Gill Nets.

No data are available on the selection of groundfish by gill nets in the ICNAF area.

Traps.

No experimental evidence is available with which to make estimates of the selection by traps, Some general knowledge of the use of traps and their catches is presented in Table 7.

Danish Seines.

European experiments have shown that seine net escapement is usually higher than otter trawl escapement for equivalent mesh sizes. Since Danish seining is of little importance in the ICNAF area and species caught differ from those caught in European waters, no estimates have been attempted from the European information.

TABLE 7. Information regarding catches and selectivity of traps.

Species	Where Traps Used	Sizes Caught	Selection
Cod	Extensively for inshore fisheries of Subareas 2 and 3 and Subdivisions 4R and 4S. Also used in Subarea 1.	For example, off Nfld. east coast 1950-53, 14% by number under 45 cm; 54% by number under 55 cm.	About 15-20% by number within selection range of $4\frac{1}{2}''$ mesh; more than 50% within selection range of $5\frac{1}{2}''$ mesh. However, since traps take the smallest fish in the area, there is no quantitative evidence re possible escapement.
Haddock	Small catches in Subarea 4.	Vary; in 1928 less than 2% under 45 cm, 30% under 55 cm in 1955—none of scrod size (under 50 cm)	Catches mainly large haddock. General knowledge of the area suggests only large fish present; thus scarcity of small fish in the catch not a result of escapement.

FURTHER RESEARCH REQUIREMENTS

Any evaluation of the adequacy of our current knowledge of gear selection must be related to the importance of species and gears. To this end we have presented in Table 8 a summary of landings from the ICNAF area for 1955. The most important species is cod; followed by haddock; redfish; and flounders, of which the American plaice is the leading species. The relative importance of species varies somewhat from subarea to subarea.

TABLE 8. Statistics of landings from the ICNAF area, 1955, in thousands of metric tons.

Species	SUBAREA					
	1	2	3	4	5	Total
Cod	265	26	429	159	12	892
Haddock	—	—	105	43	51	198
Redfish	32	—	18	60	14	123
American Plaice	—	—	15	12	2	60
Witch	—	—	3	7	1	
Other Flounders	1	—	1	2	16	
Halibut	1.4	—	1	1.8	—	4
Pollock	—	—	7	19	10	36
Silver Hake	—	—	—	—	46	46
Other	4	—	2	17	107[a]	130

[a] Includes menhaden, herring, alewives, etc.

Summaries of the catches by various gears are given in Tables 9 and 10. Otter trawls are of the greatest overall importance for cod, particularly those of trawlers over 500 tons. However, hooks and traps are also of considerable importance, especially in the inshore fishery. For the remaining species, otter trawls, primarily those of trawlers under 500 tons, are most important.

We are lacking information on the catches of many important species by certain gears.

TABLE 9. Landings of major species by different gears from the ICNAF area, 1955, in thousands of metric tons.

Gear	Cod	Haddock	Redfish	Flounders
Otter trawls				
over 500 tons	345	56	32	—
151-500 tons	28	77	91[a]	56[a]
under 151 tons	30	48		
Pair Trawls	33	4	—	—
Traps	ca. 100+	Some	—	—
Hooks (Longline and Handline)	ca. 300+	ca. 12	—	0.3
Seine Nets	Negligible	Very few	Very few	1.3
Gill Nets	Negligible	Few	—	—

[a] Categories "151-500 tons" and "under 151 tons" combined.

TABLE 10. Landings of minor species by different gears, from the ICNAF area 1955, in thousands of metric tons.

Gear	Halibut	Pollock	Silver Hake
Otter Trawls	0.8	Some pollock caught by most gears in Subareas 3, 4, and 5.	46
Hooks	1.7		—
Miscellaneous	1.7[a]		—

[a] Mainly hooks

The best summary of research needs in gear selection is given in the "Outline of Present Research and Long-Range Needs in the Convention Area" prepared by the Research and Statistics Committee in 1956. Although some progress towards filling the indicated gaps in our knowledge has been made since preparation of this document, it still summarizes current essential needs in gear selection research. The following excerpt contains the two sections relating directly to gear selection:

"(1) Determination of the size selectivity of different kinds and sizes of fishing gear.

Present Status: Selectivity of the catching gear is unknown for about 80% of the cod taken in the area. More adequate knowledge is available for about 75% of the present haddock catch.

a. Otter trawl selectivity—Much research has been carried out with single, large mesh codends by several countries. Selection experiments with multiple codends have not been attempted.

b. Pair trawl selectivity—Inactive.

c. Hook selectivity—Canada has compared the selectivity of various sizes of hooks. Norway has compared the sizes of fish caught by hooks with the sizes of fish caught by other gears.

d. Trap selectivity—Inactive.

Additional Resources Required:

a. Mesh experiments with traps should be carried out by countries using these gears to determine what effect mesh size has on the size of the fish caught.

b. Selective properties of otter trawls with multiple cod ends should be obtained by the countries using these gears. Covered net experiments and comparative fishing experiments are recommended.

c. Present knowledge about hook selection should be assembled and considered in relation to mesh selection. This would provide a basis for planning further hook selection experiments.

d. Length composition samples of cod and haddock taken by different gears should be compared to help assess the general problem of selectivity. For each sample a complete description of the catching gear must be included.

(2) Determine which parts of the trawl and which sections of the codend contribute most significantly to the release of fish. Determine what factors cause variation in mesh selectivity, such as effect of catch size, towing time, towing speed, differences between material, etc.

Present Status: In the ICNAF area a number of mesh selection experiments have been carried out by Canada and the U.S. Experiments are continuing on the differences in selection for different materials. Many of the experiments carried on by other countries outside the ICNAF are are applicable.

Additional Resources Required:

Most of the mesh selection experiments carried out in the ICNAF area have been only summarily analyzed. All extant mesh selection data should be analyzed with emphasis given to determining the causes for variation in selection results and amount of escapement in forward parts of the trawl. Results of the analysis should be presented as a basis for determining what further mesh selection experiments are needed. Because future planning is so dependent on the consideration of these past experiments, this should be carried out as soon as possible. Publication of the results by ICNAF should be considered."

Good progress has already been made in carrying out the goals of (2) above. This was accomplished through the workshop gathering of American and European gear selection workers at the Joint Meeting of ICNAF, ICES, and FAO in Lisbon during May and June of 1957. The collection of experimental results presented at the meeting is scheduled for publication in 1959.

APPENDIX I: GENERAL BACKGROUND MATERIAL

How Gear Selectivity is Measured.

The selectivity of gear is determined usually by either capturing the fish which have escaped the gear (covered codend experiments) and comparing their sizes with those retained by the gear, or comparing the catches of different gears fished at the same time and place. These comparisons usually reveal at first a slow increase in the retention of fish from the smallest size at which some are first retained, followed by a rapid increase in retention, and finally reaching a stage of only gradually increasing retention to the point where full retention is achieved. When such data are plotted they take the form of an extended "S-shaped" or sigmoid curve, the "selection curve" for the gear. A typical selection curve is shown in Figure 1.

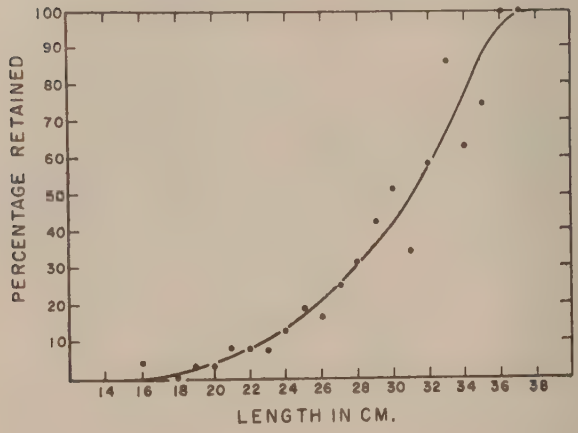

Fig. 1. **Percentage of redfish of each length retained by a manila codend of 132 mm mesh size. (From data of Clark 1957e).**

Variations from the S-shaped pattern are found for certain gears such as gill nets, in which the retention increases to its highest value at a certain fish size and then decreases again past that size, thus describing a bell-shaped or normal curve.

How the Selectivity Process is Defined.

Selection of the type that takes place in otter trawls is defined by various properties of the sigmoid selection curve. These properties are usually the lengths of fish retained at various points along the curve, such as the 25, 50, and 75% retention points. Selectivities of different gears and sizes of gears are most often described simply by giving their 50% retention lengths (the length at which 50% of the fish are retained by the gear and 50% escape). The selection span (distance between 25 and 75% retention lengths) is often included to give a measure of the steepness of the curve. The "regression coefficients" are often used to describe the relation between 50% retention length point for various gears and average internal size of mesh (see Appendix III for illustration). If the form of this relation has an intercept of 0, the "b" value of the regression formula is sufficient to define the relation. This value, termed the "selection factor," is thus the quotient of 50% retention length/mesh size, a higher factor indicating greater escapement (Permanent Commission, 1956). Selection factors for otter trawls, for example, will vary from 2.0 to 5.0, depending principally upon the species and material from which the netting is made.

The Range of Fish Sizes over which a Selection Process Extends.

The selection process is not precise, but as shown in Figure 1 operates over a more or less extended range of fish sizes. For otter trawlers it has been found experimentally that meshes over 150 mm select sizes of haddock over a range of 35 cm or more. This range, called the selection range, which extends from zero to one hundred percent retention has been related to the size of the mesh. The range is very much smaller for smaller sizes of mesh; for instance, reduced to about 8 cm for a mesh of 75 mm for haddock.

Difficulties Arising from the Extended Selection Range.

Although the gear operates selectively over a large range of sizes, the greatest selection occurs

within a much narrower range of sizes. Most investigators consider the important part of the selection range as that occurring within the "selection span". The haddock selection span, for instance, may be as small as 4 cm in 75 mm meshes and as great as 14 cm in meshes of 150 mm or over. This means that to obtain substantial escapement of a certain size of fish one must accept the escapement of a substantial quantity of fish over those sizes. For example, if one is to obtain 75% encapement of 34 cm haddock from a trawl net, one must allow about 60% escapement of the fish at 36 cm, 40% at 38 cm, 25% at 41 cm, and smaller percentages of the larger sizes. This factor must be taken into consideration in recommending mesh sizes for fisheries to be sure that proper allowances are made.

Effect of Experimental Error.

The variations in gear selection results arise from experimental and from substantive causes. As an example of the kind of experimental error encountered, the use of a small mesh cover over the codend may easily result in a dampening of the escapement if the cover is not properly fitted. Results from replicate tows with large and small mesh nets tend to give higher escapement values than those from covered codends, as observed by Saetersdal (1957a) and Clark (1957g). This presumably comes about through differences in the catching rate of the two gears for different sizes of fish which are not due to the actual selectivity of the meshes. The greatest problem in comparing results of various experiments is that of isolating the causes of variation and attempting to find reasons therefore.

Selective Properties of the Trawl for Different Species.

Marked differences in escapement values have been shown for various species, although the reasons for this are not well understood. The relation between fish shape and mesh shape is undoubtedly important. Flatfish, for example, do not escape nearly so well in respect to their length as roundfish. This could be expected

because they are so much broader. Redfish, which have a relatively low girth factor (length / girth ratio) compared to other roundfish are also characterized by low escapement in respect to length. Also there may be important differences among various species of the same family. Cod, for example, escape in somewhat greater numbers at any size than haddock. Silver hake which should, because of their high girth factor, escape in greater numbers than haddock, actually do not. This means that selection has to be determined separately for each species.

Escapement of Fish from Forward Parts of the Net.

It has been accepted that most of the escapement from otter trawls takes place in the codend. However, recent data indicate that although this is true in general, appreciable numbers of some species escape through the forward parts of the net. This escapement is usually restricted to smaller sizes of fish than escapement through the codend.

Data from experiments in the ICNAF area have shown, for instance, that nearly 100% of the very smallest sizes of haddock and silver hake escape through the forward parts of the net (Clark 1957c). A considerable, but gradually reducing, amount of escapement takes place toward the larger sizes in normal sigmoid fashion. In relation to the area of netting available, however, escapement through the forward parts is quite low as these parts contain 50 to 100 times as many meshes as the codend. Furthermore, for haddock the 50% point occurs at a lower fish size for the forward netting than for the codend, with the same mesh size in each.

It has been demonstrated that fish do not escape equally throughout various parts of the codend, but rather escape through the first few rows of clear meshes ahead of the accumulated catch (Beverton, 1957b; and Clark 1957b).

Differences in Escapement Associated with Differences in Net Material.

The most important substantive (non-experimental) sources of variation in selection

work are those associated with the type of material of the nets. At equivalent mesh sizes, meshes of lighter, more flexible twines usually give higher escapement than those of heavier and stiffer twines. For example, the 50% retention length for haddock is about 5 cm higher for a single cotton than for a double manila codend at a mesh size of $4\frac{1}{2}$ inches (Table 3). Similarly, for silver hake it has been shown to be about 6 cm higher for light single nylon than for single manila at a mesh size of 4 inches (Table 5).

Effect Upon Escapement of the Speed with which the Net is Towed.

Little direct evidence is available on this subject because few controlled experiments on the effect of different towing speeds on escapement have been conducted in the ICNAF area. However, some of the differences in escapement between large boats and small boats (which tow more slowly) are probably due to differences in towing speed. Experimental trawling for selection purposes is usually carried out at a towing speed approximating commercial practice so that results will be directly applicable. However, "commercial practice" varies from fishery to fishery and area to area, making it difficult at times to compare and utilize the results of various experiments.

Effect Upon Escapement of the Length of Time During which the Net is Towed.

That longer tows appear to afford greater escapement has been documented for the ICNAF area in controlled haddock escapement experiments in which the 50% retention length increased by more than 5 cm in one series of tows when towing time was increased from 20 to 80 minutes (Clark 1957a).

European work has not altogether confirmed this, although there is some suggestion in Gulland's (1956) results that 3 hour tows may give higher escapement than $1\frac{1}{2}$ hour tows. No difference is apparent in the results of Parrish and Pope (1957).

Such differences as may be found will not be so large as to interfere greatly with comparison of experimental results or with their application, since tows usually approximate commercial practice, which is most often 60 to 120 minutes in the ICNAF area. The difference in 50% retention lengths between these extremes should be only a centimeter or two.

Size of Catch in Relation to Escapement from Codends.

Many investigators have shown that larger catches are generally associated with lower escapement. Work in the ICNAF area has shown that the codend 50% point for haddock, for example, may decrease 5 cm from light to heavy catches. The expected catch level in a fishery should thus be considered in planning mesh control programs.

Escapement from the Trawl Net Under Tow.

It has been amply demonstrated that meshes of the net remain open during towing and that fish escape during this time, not just while the net is being hauled. Davis (1934), for instance, demonstrated with a special choking device on the codend cover that 90% of the fish escaped while the net was under tow. Davis's findings have subsequently been confirmed by observations of SCUBA divers in shallow water and underwater television in deeper water.

Whether the fish escape undamaged or suffer injuries, perhaps, mortal, in escapement has not been adequately answered. Examination of limited results from tagging of escapees taken in codends and covers has not indicated any greater mortality for those which passed through the codend meshes into the cover. The SCUBA and underwater television observations have shown fish to swim away very vigorously, and apparently unhurt, after escaping from codends under tow. It is, of course, possible that the escape effort could result in damage having some later consequence.

Experimental Results Compared with the Results of Large Mesh in Practice.

Davis (1934) compared catches with large and small mesh on two commercial trawlers and demonstrated that selection experiments were borne out in practice. Clark's study of the regulated Georges Bank fishery has shown selectivity of the $4\frac{1}{2}$ mesh for haddock to be almost exactly that predicted by the experimental evidence.

Increased Efficiency of Large Mesh Otter Trawls.

It is a common result in comparison of catches of large and small mesh gears to find that larger meshes catch more fish of the larger sizes. This was first shown by Davis (1934) who showed that the large mesh caught 11% more fish of sizes beyond the selection range. Perhaps the best demonstration is that for the Georges Bank haddock fishery, in which the regulated ($4\frac{1}{2}$ inch mesh) trawlers caught 9% more fish of larger sizes than the small mesh ($2\frac{7}{8}$ inch) study group vessels (Clark and Nichy 1957). These increases are noticeable particularly for sizes of fish just beyond the selection range of the net.

The Value of Special Trawl Gears Devised for Improving Escapement.

Various types of special trawls or modifications of trawls have been devised with the hope of improving the precision of the selective action of the net. These trawls, incorporating rigid meshes of wire, special "escapement panels", or meshes hung in a special fashion to insure their being open, have not shown sufficient superiority over the standard type of netting to be considered for introduction into the fisheries.

APPENDIX II: HADDOCK SELECTION

So much data are available from codend selection trials with haddock that time did not permit an exhaustive analysis of the causes of variation. The lines of best fit plotted in Figures 2 to 5 were calculated by the least squares method

from 50% escapement lengths for various mesh sizes. In Figure 3, the data of "early U. S. trials" were those of Herrington (1935). "Early European trials" were those reported by Bowman (1928), Davis (1934), Russell and Edser (1926), and Todd (1911). The data of Parrish are partly from the Lisbon papers (with Pope 1957) and partly from personal communication. "Other recent European trials" consist of data from the Lisbon papers of Beverton (1957a), Margetts (1957), Saetersdal (1957b), and v. Brandt (1957). The data shown in Figures 2, 4, and 5 are from the Lisbon papers of Clark (1957d), Margetts, McCracken (1957c), and Templeman (1957a). The category "European" in Figure 5 includes the data of Margetts and v. Brandt.

The line for manila (Figure 2) was fitted to the results of 30 separate trials. Only those results for 45/4 and 50/4 twine were used in the least square calculation as these are the common sizes in use in the ICNAF area. Inclusion of results for 75 and 125 yard twines would affect the line very slightly, however. The European and early U.S. experiments are included in Figure 3 for comparison. The European experiments vary considerably but in general confirm the ICNAF line. The early U.S. experiments, based on alternate tows and "trouser" codend methods, are now not considered to be applicable because of the difficulty of translating Herrington's mesh measurements into those presently

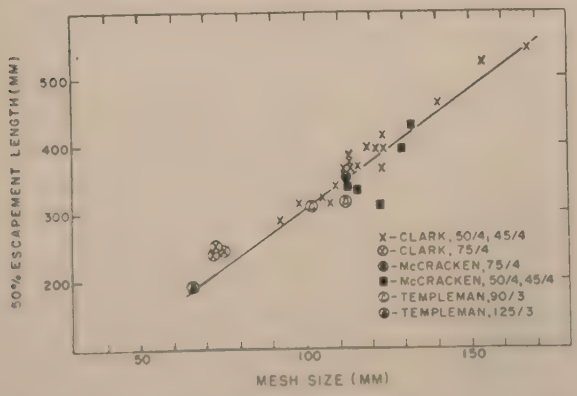

Fig. 2. **Haddock 50% retention lengths for double manila for the ICNAF area. Line fitted to 45/4 and 50/4 data only.**

used and because of the inherent bias in the experimental methods used.

The line for manila, when extrapolated, does not pass through the zero point. For this reason the selection factor is not constant, but an average factor of 3.2 is roughly applicable to the middle range of values and will suffice for most purposes. Nor does the line of best fit for single cotton (Figure 4) pass near the intercept. The one European point (not included) would have the effect of drawing the intercept somewhat nearer to zero.

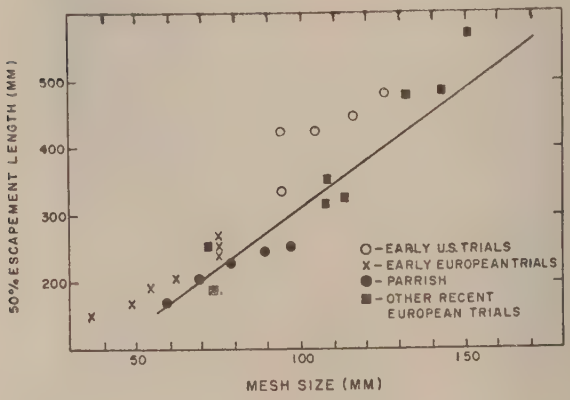

Fig. 3. **Haddock 50% retention lengths for double manila for the European area, with the ICNAF line (from Fig. 4) drawn in for comparison.**

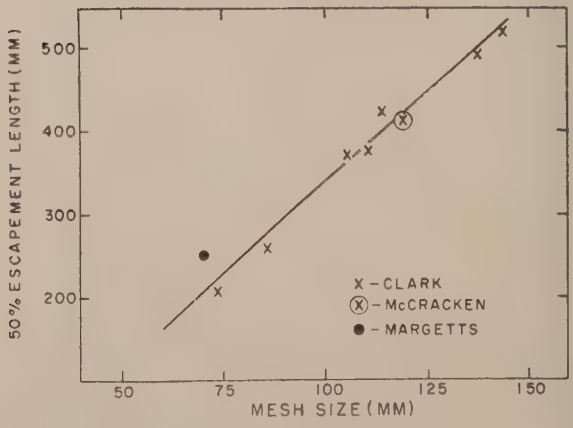

Fig. 4. **Haddock 50% retention lengths for single cotton.**

The data for synthetics are much more variable (Figure 5). All the synthetics are considered together since so few data are available for the individual types. Final consideration of differences in various kinds of twine must await more detailed analysis. One factor of considerable importance is that synthetic codends often have much larger meshes at their after ends, past the splitting strap, due to the stretching effect of the weight of fish being hauled aboard. This will produce an effect of higher 50% retention lengths for given average mesh size of the whole codend, since most fish escape from the area behind the splitting strap. If the 50% length is plotted against the mesh size in the after quarter only, for the U. S. experiments a somewhat better fit is obtained. For the purpose of making our estimates we have fitted the line in Figure 5 to all data on the basis of the average mesh size for the whole codend.

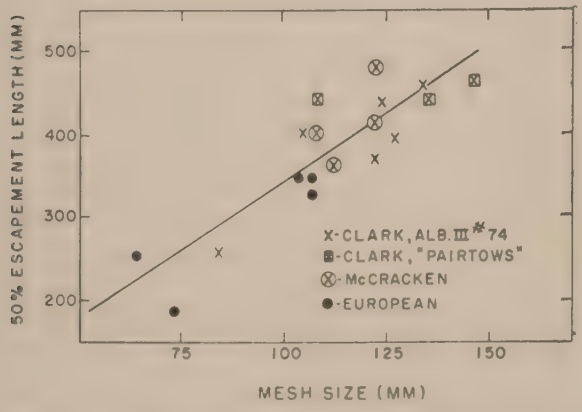

Fig. 5. **Haddock 50% retention lengths for all synthetics.**

APPENDIX III: REDFISH SELECTION

The data used for the following analysis have been taken mainly from the Lisbon papers of Clark (1957c), McCracken (1957b), Saetersdal (1957b), and Templeman (1957b). All the data except those of Saetersdal are for the American form of redfish tentatively referred to as *Sebastes marinus mentella*. Saetersdal's selection data are for *Sebastes marinus marinus*. Saetersdal's mesh sizes were measured under 8 lb. pressure,

while the remainder of the mesh size data are for 12 lb. pressure. In all cases the codend meshes were of double manila twine. All the redfish measurements were from the chin, with the mouth closed, to the end of the median ray of the caudal fin.

All the alternate tow data have been adjusted by assuming that large redfish, those beyond the size of 100% retention by the codend meshes, were present in good numbers in both large and small meshed nets and by adjusting the remaining part of the frequency accordingly.

In the alternate tow work the large mesh nets had codend, lengthening piece, and after belly meshes of the large mesh, while the remaining forward parts were of the same mesh size as forward parts of the small mesh nets (usually 6 inch mesh overall, apart from the usual gradation of mesh sizes from the forward belly to the size used in the after part of the belly).

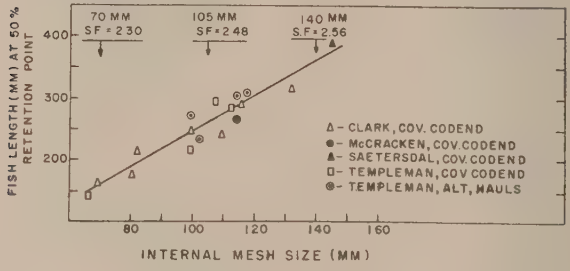

Fig. 6. **Redfish 50% retention lengths for double manila codends, 45/4 - 125/3.**

Figure 6 indicates that the selection factor for redfish increases with mesh size; e.g. from 2.30 at a mesh size of 70 mm to 2.56 at a mesh size of 140 mm. The factor for 75% retention also increases with mesh size, from 2.54 at a mesh size of 70 mm to 2.75 at a mesh size of 140 mm (Figure 7) as would be expected.

Figure 8 shows that the escapement for 45-50/4 manila, for which data are available only at the larger mesh sizes, is usually lower than that for 90-100/3 manila. It is thus possible that if heavier twine had been used at smaller mesh sizes the selection factor would have declined even more rapidly.

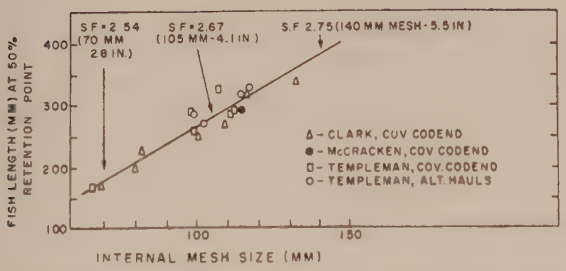
S.F.=2.54 (70 MM 2.8 IN.)
S.F.=2.67 (105 MM-4.1 IN.)
S.F. 2.75(140 MM MESH-5.5 IN.)
Δ - CLARK, COV CODEND
● - McCRACKEN, COV CODEND
□ - TEMPLEMAN, COV. CODEND
○ - TEMPLEMAN, ALT. HAULS
FISH LENGTH (MM) AT 50% RETENTION POINT
INTERNAL MESH SIZE (MM)

Fig. 7. **Redfish 75% retention lengths for double manila codends, 45/4 - 125/3.**

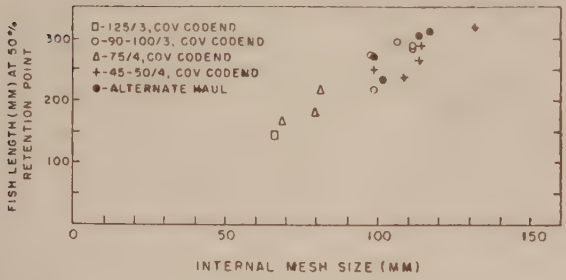

Fig. 8. **Redfish 50% retention lengths for double manila codends of different twine sizes.**

The data of Clark and Templeman have been further analyzed to show that in the covered codend experiments (Figure 6), the selection factor is lower with larger catches and higher with smaller catches.

Most of the selection points in the figures were obtained from experiments in which there were small to moderate catches. Selection factors are needed for larger hauls, preferably from the paired tow method, or if this is not possible, from alternate tows by commercial vessels with different mesh sizes operating in areas where large catches are obtained.

APPENDIX IV: COD SELECTION

Within the ICNAF area cod are caught in quantity by the greatest variety of gears, which include otter trawls, hooks, traps and pair trawls.

Results of selection experiments with hooks of different sizes have been taken from McCracken (1957a). Comparisons of sizes of cod caught by longline and otter trawl have been derived from

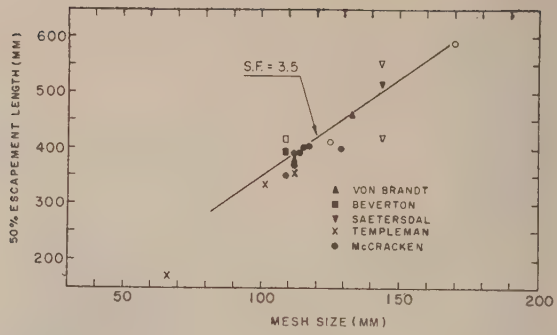

Fig. 9. **Cod 50% retention lengths for manila codends of double strand construction. Open symbols represent results from experiments with less than 50 fish per tow in the selection range.**

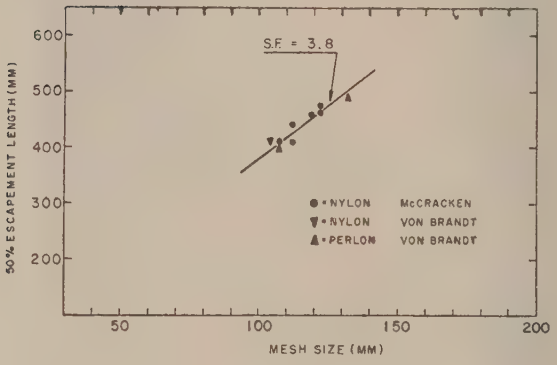

Fig. 10. **Cod 50% retention lengths for nylon codends of both double and single strand construction and perlon codends of double strand construction.**

McCracken (1957a) and Saetersdal (1957c). Comparisons of sizes of cod caught by handlines and traps have been taken from Templeman and Fleming (1956).

In many cases the otter trawl selection results for cod have been a by-product of selection experiments for haddock. Data are available only for a fairly narrow range of mesh sizes and experiments have not been carried out with small mesh codends.

Results of covered net experiments with manila codends show a selection factor of about 3.5 for cod, Figure 9. A comparison of the length composition of cod caught by large and

small mesh manila nets in the commercial fishery of Subarea 4, suggests a similar selection factor. Results of covered net experiments with nylon and perlon codends show a selection factor of about 3.8 for these materials, Figure 10. Selection experiments for cod with cotton codends have included only two mesh sizes (102 and 119 mm). These experiments suggest that cotton has a selection factor of about 3.7, intermediate to that of manila and synthetics.

The selection span, as described by the distance between 25 and 75 percent retention lengths is quite variable, Table I. Results for manila codends indicate that selection span increases with mesh size. The selection span for manila codends also appears to be greater than for nylon codends of similar mesh size. For purposes of this report a selection span of 10 cm has been chosen as most representative.

TABLE I. Selection spans for cod in covered net experiments with manila, nylon and cotton codends.

Selection Span	Codend Material and Frequency		
cm	Manila	Nylon	Cotton
6	2	3	—
7	1	1	—
8	2	1	2
9	2	—	—
10	6	—	—
11	2	—	—
12	3	—	—
TOTAL	18	5	2

APPENDIX V: SILVER HAKE SELECTION

The only extensive codend selection trials for the ICNAF area were conducted aboard a small (47 gross tons) otter trawler, *Priscilla V.*, of the type which carries out the major part of the silver hake fishery (Clark 1957f). The trawler towed a small net slowly (2½ knots) and results may not be directly applicable to larger trawlers. Examination of these data (Figure 11) indicated much duller and much lower escapement than would be expected from the girth of silver hake. Higher escapement is indicated by experiments on the related European hake. The relation between mesh size and 50% escapement length is fitted better with a curve than a straight line.

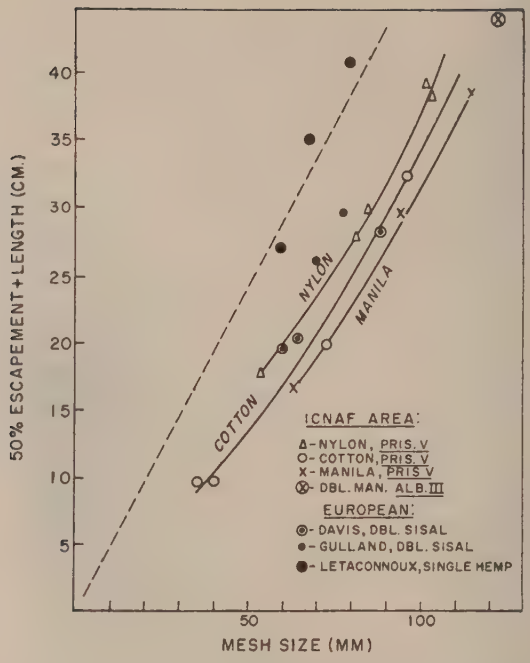

Fig. 11. **Silver hake 50% retention lengths and mesh size. The dashed line represents mesh circumference = fish girth (for *M. bilinearis*).**

The curves for the three different materials are of analogous shapes. The line for cotton may be extrapolated back to the zero point. It is perhaps reasonable to assume that the other two curves might also be fitted to conform to a zero intercept. However, consideration of the significance of intercepts is beyond the scope of this study and the estimates of 50% retention lengths have been interpolated from the curves shown which have been fitted as closely as possible to the data for each material.

The single point for double manila (*Albatross III*) was not used in drawing the line as a specific 50% point could not be estimated from the sparse data. These data do, however, indicate a 50% retention length of a minimum of 450 mm for the 123 mm mesh, which is somewhat higher than would be indicated from the *Priscilla V.* work. More selection trials must be carried out with the heavier gear and faster towing speeds

of larger trawlers to ascertain whether, in fact, they would provide greater escapement.

European data, as summarized by Gulland (1956) and giving higher selection, are indicated in Figure 11. The data of Davis (1934) coincide well with the ICNAF data. Gulland's own data give higher, but not unreasonable, estimates.

The data of Letaconnoux (1955) are so much higher, notwithstanding the fact that they are for single twine of hemp, as to be considered inappropriate for our use. These data are for replicate tows which seem usually to yield higher escapement estimates than those for covered codends. The data of Letacconoux show half the hake escaping of a size which, on the basis of data for silver hake in the ICNAF area, are larger in girth than the mesh is in circumference and theoretically would be too large to pass through the meshes (see dashed line in Figure 9). Moreover, this is only the 50% length, indicating that many even larger fish are escaping. Notwithstanding the fact that the mesh size is only an average and some larger meshes are undoubtedly present, such escapement estimates remain extraordinarily high and more intensive examination of Letaconnoux's data would be required before they could be considered appropriate for our purposes. As such extensive analysis is beyond the scope of this report, the European data have not been utilized for our estimates.

APPENDIX VI: FLATFISH SELECTION

Otter trawl mesh selection results for two species of flatfish, American plaice and witch, are presented in Table 5, p. 86. These data have been obtained as by-products of selection experiments for various roundfish species. The data for American plaice are presumably better than those for witch, since they were caught in larger numbers.

These results suggest a selection factor for American plaice of about 2.0 with manila codends and 2.2 with nylon codends. It is probable that the selection factors for witch are slightly

higher than for American plaice. Selection factors of the above order are similar to those found for the European plaice from extensive experiments carried out in the North Sea.

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PART 3

Summaries of Research 1957

A. Summaries by Countries

I. Canadian Researches, 1957

2. SUBAREA 4

By W. R. MARTIN

Canadian research in the offshore waters of Subarea 4 is carried out by the Fisheries Research Board of Canada and the Quebec Department of Fisheries. The Board's Station at St. John's Newfoundland, did redfish work in Subdivision 4R. The Quebec group studied redfish in Sub-Division 4S and cod in the northern part of Subdivision 4T. The Board's Station at St. Andrews, New Brunswick, investigated cod and haddock in Subdivisions 4T to 4X, and the Atlantic Oceanographic Group at St. Andrews continued hydrographic work throughout the subarea. This report summarizes the various Canadian researches in Subarea 4 which are considered to be of interest to the Commission.

Statistics and sampling of commercial landings are reported separately. During post-war years the numbers of offshore otter trawlers and longliners have steadily increased. This has increased the work involved in relating landings to area of capture and fishing effort. Sampling of commercial landings included approximately 15 thousand cod and 14 thousand haddock measurements, and age determinations for about one fifth of these. Another 14 thousand cod were measured on commercial draggers in order to determine the proportion and sizes of cod discarded at sea.

Haddock, *Melanogrammus aeglefinus* (L.). The haddock program has been carried out in co-operation with the United States laboratory at Woods Hole. In 1957 this research included

tagging, exchange of statistics and wharf sampling data, and development of the otolith method of age determination for all Subarea 4 haddock.

In March and April, haddock were caught by otter trawl from depths of 45 to 80 fathoms in the LaHave-Browns region of Subdivision 4X by the M.V. *J. J. Cowie*, and 1123 of these were tagged. In November and December, 1085 haddock were tagged at the mouth of the Bay of Fundy in Subdivision 4X. Most of these were tagged from the same research vessel in Passamaquoddy Bay and Grand Manan Channel over depths of 20 to 60 fathoms. Early returns are consistent with results of earlier taggings which show a southern movement of haddock to offshore grounds in winter, and a northern movement to inshore grounds in summer months. The success of tagging experiments is shown by the high returns from earlier taggings. Over 40% of the disk-tagged haddock released off Lockeport, N. S., (Subdivision 4X) were recovered by the end of 1957. Recaptures in 1957 were still 6% of the possible number of tagged fish remaining in the water at the end of 1956, without any allowance for natural mortality since tagging.

Haddock statistics and sampling data for Subdivision 4X were sent to Woods Hole for analysis. United States statistics and sampling data for Subdivision 4V and 4W were examined at St. Andrews. A large 1952 year-class was largely responsible for increased landings of haddock from Subdivision 4W in 1956, and this year-class continued to be dominant in 1957 catches from Subarea 4. Growth of this year-class appeared to be normal for the Western Bank region of Subdivision 4W.

As a result of an earlier conclusion that otoliths were more satisfactory than scales for age determination of Subarea 4 haddock, all age analyses are now based on otolith readings.

Cod, *Gadus callarias* L. Cod research, in 1957, was concentrated in Subdivision 4T. Investigations included tagging, parasite incidence, growth experiments, models of the fishery, effects of mesh regulation, mesh selection, and a new census program.

In late July and early August, 1202 cod were tagged at the Magdalen Islands at depths of 12 to 26 fathoms. Most of these cod were caught by handline from the M.V. *J. J. Cowie*. First-year recaptures showed a seasonal pattern of migrations similar to that observed earlier for cod tagged farther north off Shippegan Island. Summer recaptures were mainly taken near the Magdalen Islands. Winter recaptures were taken farther south in deeper water, outside the Gulf of St. Lawrence. Percentage recaptures have been smaller than the high returns from earlier taggings (Table I).

Studies of parasite incidence contribute to definition of cod populations. The proportion of cod infected with *Porrocaecum decipiens* (Krabbe), *Lernaeocera branchialis* (L.), and *Clavella uncinata* (O.F. Muller) varied with the size of fish and with the area of capture.

Returns of fish lengths with recaptured tags from 1955 and 1956 cod tagging experiments have provided direct measurements of growth. Results from the 1955 tagging show that the average growth increased from 0.6 cm in July to 3.7 cm in November of the year of tagging, from 5.3 to 7.7 cm during the summer of 1956, and from 9.3 to 11.3 cm in the summer of 1957. There was considerable growth during winter months. The results from the 1956 tagging showed similar changes, but it was evident that there was more growth from 1955 to 1956 than from 1956 to 1957. This difference between years was also observed from otolith studies of commercial samples.

Data on growth, mortalities, fishing intensity, and size at first capture have been used to construct models of the Subdivision 4T cod fishery. The models provide information on fishing conditions required to make best use of the resource. On the basis of present models it is predicted that the sustained yield can be increased by increasing fish size at first capture with a minimum mesh size of at least $5\frac{1}{2}$ inches, and by limiting the total amount of fishing effort to a level approaching the present intensity. The evidence was presented to the Commission in 1957.

TABLE 1. Subarea 4 Cod Tagging Experiments 1953-57.

Year tagged	Place	Type of tag	Method of Capture	No. tagged	Percentage Recaptured					
					Calendar year of tagging	2nd year	3rd year	4th year	5th year	Total re-coveries
1953	Lockeport 4X	Disk	Handline	608	34	27	5	3	2	62
1954	Louisbourg 4V	Disk	Handline	492	21	21	17	5	—	51
1954	Canso 4W	Disk	Handline	299	25	29	20	3	—	59
1955	Northern N. B. 4T	Disk	Handline	2650	7	14	8	—	—	27
1956	Northern N. B. 4T	Disk	Handline	482	9	24	—	—	—	30
1956	Northern N. B. 4T	Disk	Otter Trawl	922	7	9	—	—	—	16
1956	Cape Breton 4T	Disk	Otter trawl	733	10	6	—	—	—	16
1957	Magdalens 4T	Disk	Handline and otter trawl	602	2	—	—	—	—	
1953	Lockeport 4X	Hydrostatic	Handline	933	24	13	0.7	0.2	0.2	35
1954	Louisbourg 4V	Hydrostatic	Handline	318	13	14	7	2	—	32
1954	Canso 4W	Hydrostatic	Handline	175	26	14	3	—	—	38
1955	Northern N. B. 4T	Hydrostatic	Handline	1208	7	10	7	—	—	22
1956	Northern N. B. 4T	Hydrostatic (wire)	Otter trawl	150	10	7	—	—	—	17
1956	Northern N. B. 4T	Hydrostatic (nylon)	Otter trawl	448	8	10	—	—	—	18
1957	Magdalens 4T	Hydrostatic	Handline and otter trawl	600	3	—	—	—	—	

(Annual percentages are based on the maximum possible number of tags in the water at the beginning of the year. No allowance has been made for probable natural mortalities.)

As a first step in management of Subarea 4 groundfish the 4½-inch minimum mesh size recommended by the Commission for cod and haddock dragging entered into force for all countries on January 1, 1958. This 4½-inch mesh became effective for Canada in March, 1957. The year, accordingly, was one of transition from small- to large-mesh nets, and there was a good opportunity to compare the effects of different mesh sizes on the quantities landed and the sizes of fish caught.

The relative efficiencies of small- and large-mesh draggers have been compared from statistics of landings per trip by various size classes of New Brunswick draggers. The comparisons give no evidence of decreased landings for draggers which increased their mesh size in 1957.

There is convincing evidence that many fish are released through the larger meshes. The proportion and sizes of cod discarded at sea in Subdivision 4T were examined on 10 commercial dragger trips. As an example, three small-mesh (3-inch) trips to Bonaventure Island grounds were compared with three large-mesh (4¾-inch) trips in 1957. Discards were reduced from 31% to 12% by numbers, and from 10% to 5% by weight on large-mesh trips.

Sizes of cod caught by 3- and 4¾-inch mesh codends are compared with sizes landed by fishermen in Figure 1. It is noted that even in the larger-meshed nets the proportion of cod caught and discarded is still significant. Examination of the data indicates that a minimum mesh size of about 5½ inches would be required to eliminate waste.

New information was obtained on equivalent mesh sizes for twines other than manila. The selective properties of two twines, No. 400/3 ply twisted nylon and braided parachute cord nylon were compared with manila during covered codend studies of cod and plaice in subdivision 4T. The results confirmed earlier conclusions that nylon meshes release larger fish than manila meshes of the same size. This justifies special provisions in the Canadian regulations for nylon

twine. The results are incorporated in a gear selection review for the Commission (see page 83).

A systematic census of cod and plaice, *Hippoglossoides platessoides* (Fabr.), populations in Subdivision 4T was started in 1957 to determine recruitment, and to assess the effects of environmental factors on abundance, distribution, and movements of these fish. In May to October, 245 tows were made, 16,210 cod and 21,554 plaice were measured, and detailed observations of age, sex, maturity, parasites, and food were recorded for 3,136 cod and 771 plaice. The results should

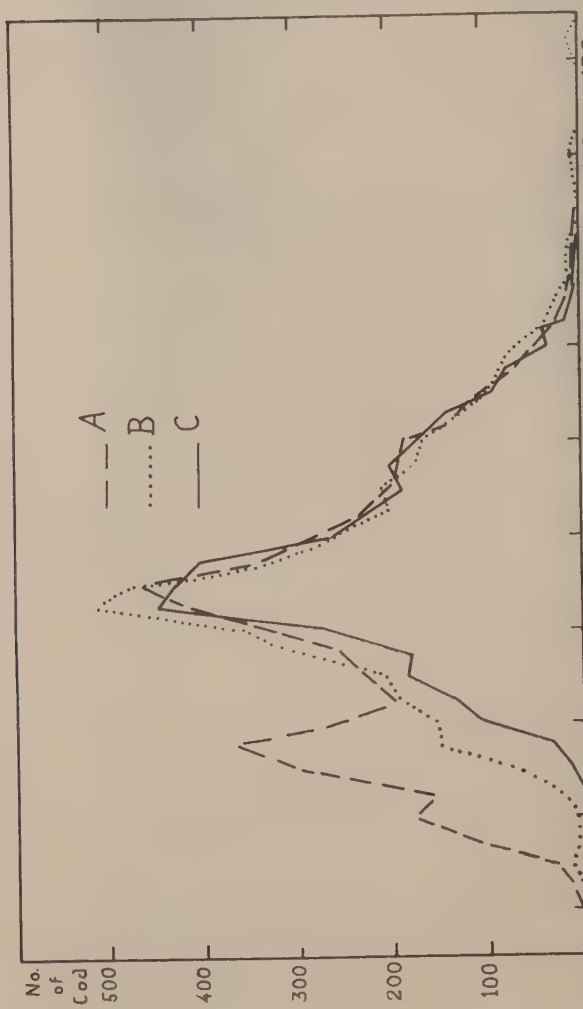

Fig. 1. Length composition of cod caught in 1957 on Bonaventura Island grounds: A — by small — (3-inch) mesh draggers (5136 spec.); B — by large — (4 ¾-inch) mesh draggers (3314 spec.); and C — landings by fishermen (2541 spec.).

lead to short-term predictions of the relative abundance of stocks available to the fishery. Preliminary results show four well defined size groups of cod. These groups changed in length during the summer, indicating growth of 3 to 6 cm. Small cod moved into Chaleur Bay at the end of June and offshore again in August.

Redfish, *Sebastes marinus* (L.). Two subspecies of redfish have been found in the central part of the Convention Area. No *marinus* -type redfish were found in an exploratory otter trawling cruise in Subdivision 4R between 100 and 180 fathoms off Port Saunders and Cape St. George; all were *mentella.* On a later cruise farther south off Cape Anguille, between 100 and 250 fathoms, of 1900 redfish only two at 120 fathoms were *marinus* type, the remainder being of the *mentella* variety.

Exploratory dragging for redfish was carried out along the south coast of Anticosti Island in Subdivision 4S, at depths of 100 to 200 fathoms. Good catches were usually taken between 140 and 150 fathoms. Over 90% of the redfish sampled were large, over 30 cm in length.

Hydrography. For the second consecutive year a winter hydrographic survey was made in the Gulf of St. Lawrence. Ice conditions were heavy in 1957 as compared to those of 1956. The production of the cold-water layer in the Gulf seems to be related to the cooling of the upper layers during the winter as well as to the oceanographic conditions of the deeper layers.

A survey of the Bay of Fundy, Scotian Shelf, and the Gulf of St. Lawrence areas was carried out in June. The hydrographic section off Halifax is described in Figure 2. In June, the cold-water layer was more developed in 1957 than in 1955. The inshore banks were presumably covered with water of sub-zero temperature. The Scotian Gulf was filled with warm water, above 8°C., and the bottom temperature on Emerald Bank varied between 5.5 and 7.5°C.

Analysis of oceanographic conditions in the Strait of Belle Isle-Esquiman Channel area indicates the development of an eddy in Esquiman Channel as a result of the dominant westward flow in the Strait and of the bottom configuration. Analysis of the properties of the deep waters in

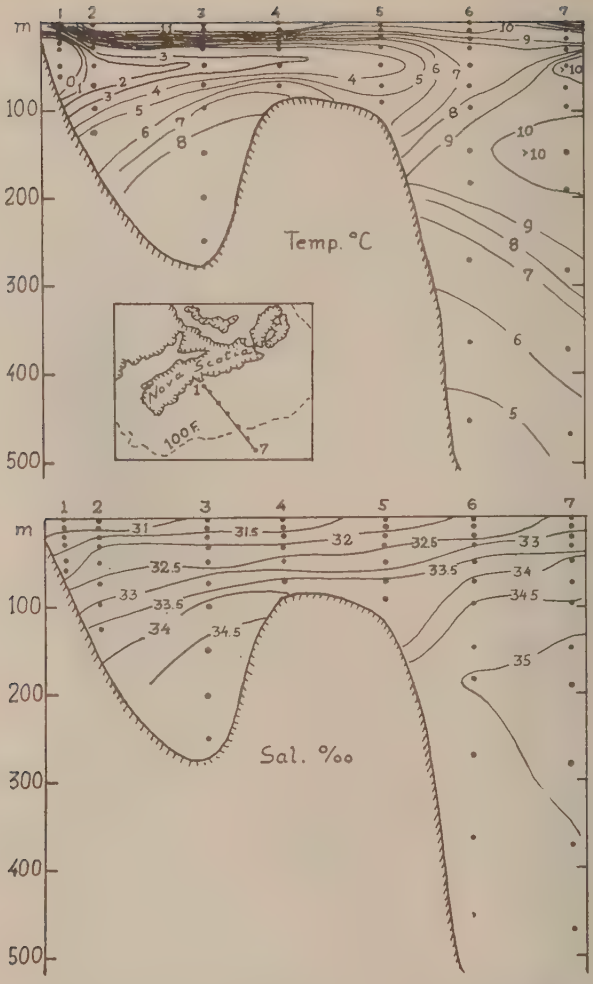

Fig. 2. **Hydrographic section across the Scotian Shelf off Halifax. 25-26 June, 1957. Above — Temperature; below — Salinity.**

the Laurentian Channel indicates warming of the deep layer from the 1920's to the 1950's. The upward trend of temperature was accompanied by an increase in the volume of the deep layer.

Analysis of surface water along the coast revealed that the cooling observed during the previous three years was followed in 1957 by a slight warming in the Bay of Fundy area and the southwestern Gulf of St. Lawrence. The Halifax area showed a decrease from the previous year.

In June drift bottles were released over the Scotian Shelf for studies of surface drift.

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PART 3

Summaries of Research 1957

A. Summaries by Countries

I. Canadian Researches, 1957

1. SUBAREAS 2 AND 3

By W. TEMPLEMAN

SUBAREA 2

Redfish were examined from various depths east of Hamilton Inlet Bank. At 158 fathoms, of 12 redfish 9 were *Sebastes marinus marinus* (L.), one doubtful, and 2 *Sebastes marinus mentella* Travin. At 170-172 fathoms 3 very large redfish were *marinus* type and 17 smaller redfish were *mentella* type, at 182-186 fathoms all 49 redfish were *mentella*. At 200, 250 and 300 fathoms all were *mentella*. Redfish were most abundant at 250-300 fathoms.

In the hydrographic section off Seal Islands, Labrador, (Fig. 1) taken on August 6 to 7, 1957, offshore temperatures in the deep water of the continental slope east of the Hamilton Inlet Bank were above 4°C. This hydrographic section has been taken yearly since 1950 and the 1957 cruise was the first occasion when temperatures as high as 4°C have been found in the deep offshore water of the area. In 1950 these offshore deep-water temperatures were 3.6 to 3.9°C.; in 1951, 3.3 to 3.5°C.; in 1952, 3.4°C.; in 1953, 3.5°C.; in 1954, 3.5 to 3.7°C.; in 1955, 3.3 to 3.6°C.; in 1956, 3.6 to 3.9°C.; and in 1957, 3.9 to 4.7°C. Salinities and inshore temperatures were generally similar to those encountered during the past few years. Some water below -1.5°C. was present near shore. This was absent in 1956 but present in 1955 and 1954. The lowest temperature encountered in 1957 was -1.67°C. and in 1956 -1.41°C.

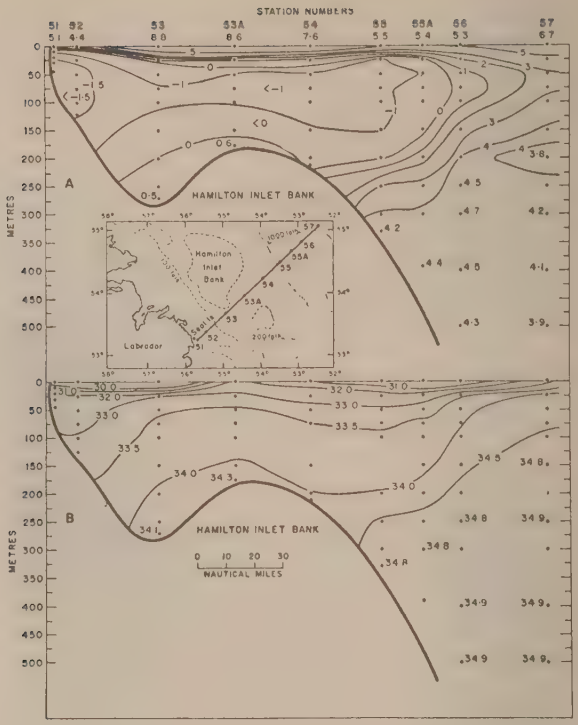

Fig. 1. **Hydrographic section off Seal Islands, across Hamilton Inlet Bank, Labrador, 6-7 Aug. 1957. A—Temperature °C; B—Salinity °/₀₀.**

SUBAREA 3

Data were collected on location of catches and catch per unit of effort of the offshore groundfish fleet. A beginning was made at gathering catch per unit of effort data in several localities for different fishing gears of the inshore cod fleet. Offshore commercial landings of groundfish were well sampled for size and age and similarly the inshore catches were sampled at Burin, St. John's and Bonavista.

Haddock, *Melanogrammus aeglefinus* (L.). During the May and June haddock otter-trawling surveys by the *Investigator II* the haddock had spread northward from their winter concentrations in the deeper water of the southwest slope of the Grand Bank to occupy most of the southern half of the bank and thus were not very abundant in any one area. On the Grand Bank as a whole

fair quantities of haddock appeared to be present. The 1949 year-class still made up a very significant part of the catch and the 1952 year-class, with some additions from the 1953 year-class and from year-classes older than 1949, made up the remainder of the haddock of commercial size. The 1950, 1951 and 1954 year-classes were completely insignificant; there was evidence of a modestly large 1955 year-class and of moderate survival of the 1956 year-class.

In the St. Pierre Bank bottom survey no significant quantities of the once very large 1949 year-class or of other haddock of commercial size were obtained. There was evidence that the survival of the 1955 year-class was only small and no evidence was found of significant survival of other year-classes from 1950 to 1954. There has been no immigration to St. Pierre Bank of the successful 1952 and 1953 Grand Bank haddock broods. There was some indication of good survival of the 1956 year-class in deep water at the western edge of St. Pierre Bank but this year-class cannot be properly assessed before it is two years old. Assuming no immigration from the Grand Bank there is at present no evidence that there will be a haddock population available for sustained commercial fishing on St. Pierre Bank for the next three years.

A study of the sound-producing muscles of the swim-bladder of the haddock has been proceeding since 1953 and is now completed. The volume of these muscles in mature male haddock at spawning time in May-June is nearly twice as great as in October-November, whereas in the mature females and in immature males and females these so-called "drumming" muscles are much smaller and there is no seasonal difference in size. Presumably these drumming muscles are used for sound production in male haddock, mainly during the spawning season, and these organs may provide a rallying sound for spawning schools. It is possible that, with suitable equipment, the sounds of these male haddock at spawning time could be an aid to identifying schools of adult haddock. These drumming muscles can be used also in sexing sexually-mature, gutted haddock, but this is not a great

problem in Newfoundland where most of the haddock are landed round.

Redfish, *Sebastes marinus mentella* Travin and *Sebastes marinus marinus* (L.). The *Investigator II* carried out deep-water explorations for redfish between 100-150 and 400 fathoms northeast of the Grand Bank and south of Green Bank. In each of these areas a few *marinus*-type redfish were found at the shallower depths while almost all the redfish, including all at the greatest depths were of the deep-water *mentella* type. The greatest redfish abundance was at 200 fathoms northeast of the Grand Bank and at 170-200 fathoms south of Green Bank. Occasional redfish were recorded to 380 fathoms. From a small commercial catch of 3000 pounds of redfish during a cod trip by the otter trawler *Blue Spray* in the southern part of the Halibut Channel between St. Pierre Bank and Green Bank in 88-90 fathoms 543 redfish were examined, of which 14% were of the *marinus* type and the remainder of the *mentella* type.

Other researches in redfish biology have been continued on age and growth, the reproductive cycle, and on a study of body proportions and meristic characters of redfish from various areas and depths. The occasional occurrence of red flesh in redfish was investigated and the pigment responsible was found to be astaxanthin.

Cod, *Gadus callarias* L. Cod investigations were chiefly on size, age, growth and year-class abundance. Sampling has been improved. A study of pre-commercial year-classes of cod was begun through the gathering of data on their abundance and the collection of material for growth studies.

Flatfishes. Age and growth studies have been continued on the American plaice, *Hippoglossoides platessoides* (Fabr.), and on the witch flounder, *Glyptocephalus cynoglossus* (L.).

Male American plaice from both St. Mary's Bay and Southern Grand Bank areas mature sexually at 5 to 11 years and females from 11 to 17 years, but, at comparable ages, for both males and females the Southern Grand Bank plaice are

much larger. Male plaice are much slower growing than females, reaching 40 cm. in length in St. Mary's Bay in 19 to 20 years compared with 15 years for the females.

On both the Southern Grand Bank and St. Pierre Bank the 1951 and 1952 year-classes of American plaice were dominant. This is interesting because both of these year-classes of haddock were failures on St. Pierre Bank, and on the Southern Grand Bank haddock survival was good for the 1952 year-class but very poor for the 1951 year-class. Plaice spawn earlier than haddock and thus their eggs and larvae encounter lower surface temperatures and different upper-water currents. Plaice also have a much more wide-spread distribution in the Newfoundland area than haddock and are much more unlikely than haddock to lose a complete year's brood over depths too great for them to survive at the bottom-seeking stage.

Hydrography. Five regular hydrographic sections usually extending from shore and across the banks to the 500-metre depth beyond the edge of the continental shelf were taken during July and August. These sections extended from off Cape Bonavista to the southern slope of the Grand Bank. Once or twice each month temperatures and salinities were taken at a station 5 miles off Cape Spear in 176 metres. Daily surface temperatures were obtained in St. John's Harbour.

In the section off Cape Bonavista there was an abundant supply of water over 4°C. in the deep water at the edge of the continental shelf. As in Labrador this is very unusual and has not previously occurred in our records at this station where the deep-water temperatures at the continental slope are generally below 4°C., similar to those we have described for the deep-water continental slope area east of Hamilton Inlet Bank. There was a considerably greater volume of water below 2°C. in 1957 than in 1956. Otherwise, temperatures in this section were little different from those of 1956 except that the 0°C. isotherm lay closer to the surface in 1957.

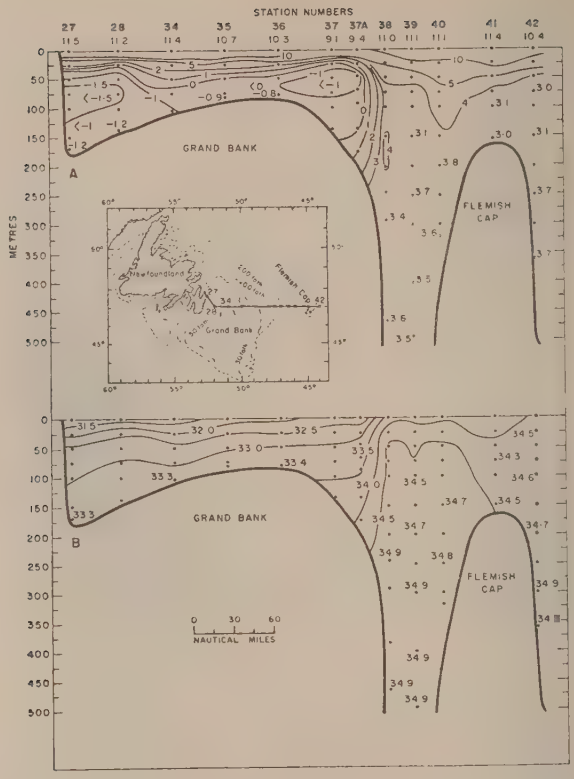

Fig. 2. **Hydrographic section St. John's-Grand Bank-Flemish Cap, 29 July-1 Aug. 1957. A — Temperature °C; B — Salinity°/₀₀.**

In the St. John's-Grand Bank-Flemish Cap section (Fig. 2) some water below -1.5°C. was present near shore. Here the lowest temperature was -1.57°C. whereas in 1956 the lowest temperature in the same area was -1.34°C. In agreement also with the very low air temperatures of the 1957 winter in the Newfoundland area, bottom water temperatures on this northern section of the Grand Bank were all between -0.8°C. and -1.1°C. whereas in 1956 bottom temperatures of 0.2°C. were to be found in this area. Temperatures east of the Grand Bank and around Flemish Cap were little different from those of 1956. The large supply of water over 4°C. which existed at the edge of the continental slope from Labrador to the northern slope of the Grand Bank did not occur in this section. The salinities of the upper

layers in the shoreward part of the section were slightly lower than in 1956.

Bottom temperatures on the southern Grand Bank in the section across the Southeast Shoal were unusually low and lower than in 1956. Bottom temperatures in the shallowest water near and on the Southeast Shoal were 0.8 and 0.1°C. in 1957 compared with 3.4 and 4.7°C. in 1956.

In the section close to and paralleling the southwest slope of the Grand Bank water below -1°C. filled most of the Haddock Channel between Green Bank and Grand Bank whereas in 1956 only a trace and in 1955 no water with this low temperature was present in this channel. Otherwise the bottom temperatures at the edge of the south-western slope of the bank in this section were very similar to those in 1956.

More warm water and water of a higher temperature than in 1956 or in 1955 was present in 1957 in the 275-metre section paralleling the southwest slope of the Grand Bank. In this section there was less water below 0°C. and considerably less water below 2°C. in 1957 than in 1956. The lowest temperatures in the Labrador Current water which occurs in the eastern part of this section were -0.7 in 1957 and -0.9 in 1956.

(Reproduced by permission from Nature, Vol. 184, pp. B.A. 79 — B.A. 80, Sept. 5, 1959)

The Benthos of Soft Sea-bottom in Arctic North America

QUANTITATIVE surveys of the benthos of soft sea-bottom were made in Baffin Island, N.W.T., during 1954 and 1955, in Greenland during 1956, and in Foxe Basin, N.W.T., during 1957, as part of expeditions organized by the Arctic Institute of North America and the Fisheries Research Board of Canada. These surveys, and others made previously in Greenland, show that *Macoma calcarea* communities[1] with standing crops generally greater than 200 gm./m.^{2} (fresh weight) occur in shallow water to about 50 m. depth in many localities in arctic North America. Other communities, some with very small crops, also occur in shallow water under localized, special conditions. They are the *Venus fluctuosa*, *Portlandia arctica* and *Chiridothea sabini* communities. The soft-bottom benthos in water deeper than about 50 m. forms at least two communities, the Foraminifera and *Arca-Astarte crenata* communities, with standing crops generally less than 100 gm./m^2.

The distribution of these communities can be related to the environmental conditions. In these arctic regions the water at depths greater than 50 m. has permanently low temperatures, which may either be just above 0° C. or below. Ekman[2] calls regions with such temperatures "low-arctic" and "high-arctic" respectively. However, overlying the deep water is an annually insolated layer, the temperatures of which may occasionally rise to 5° C. or more in summer. Low-arctic conditions can, and often do, occur temporarily at the surface over high-arctic deeper water. The two terms can apply to temperature zones arranged vertically in the sea, as well as zoogeographical regions. As such they are convenient for an ecologist to use, but their significance is mainly as a guide to the temperatures ; they imply nothing about the dynamics of the oceanographic conditions, unlike other systems of naming the arctic marine regions[3].

The *Macoma calcarea* communities have only been found where sea temperatures are permanently or seasonally low-arctic. Thus they occur in surface insolated water in the regions farthest north, but may extend deeper elsewhere, for example, Spitsbergen, Iceland[1]. They have a wide geographical range, apparently because some of the component species can adapt their breeding season to the time of year when appropriate temperatures occur, namely,

in summer in the Arctic, in winter in warmer areas. The *Venus fluctuosa* and the *Portlandia arctica* communities appear to be restricted to certain bottom sediments within the low-arctic environment : types of coarse sand and fine mud, respectively. The *Chiridothea sabini* communities have only been found in two localities, in which the most obvious common environmental factor is very low summer temperature, that is to say, permanently high-arctic. The two deeper water communities occur below the depths affected by insolation where temperatures may be either low- or high-arctic.

Scattered dredge collections from Greenland to Alaska suggest that communities of *Macoma calcarea* are very widely spread in shallow water throughout arctic North America, but are replaced by other communities in deeper water. This pattern of vertical zonation appears to be very common.

An account of the collections and a more detailed discussion of the ecological and zoogeographical concepts mentioned here is being prepared for publication. Tables listing the species collected, and their numbers and weights per grab haul, are included in a doctoral thesis[4] deposited in the Redpath Library, McGill University, Montreal.

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Fisheries Research Board of Canada,
Biological Station,
Nanaimo, British Columbia.
March 16.

[1] Thorson, G., in "Treatise of Marine Ecology and Paleoecology", 1, Chapter 17, p. 461 (Geol. Soc. Amer., Mem. 67, 1957).

[2] Ekman, S., "Zoogeography of the Sea" (1953).

[3] Dunbar, M. J., Fish. Res. Bd. Canad., Bull., 88 (1951).

[4] Ellis D. V., "Marine Infaunal Benthos in Arctic North America", Ph.D. thesis, McGill University, Montreal (1957).

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Back-pack Fish Shocker

by

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The value of small, portable, electrical fish shockers is fast becoming apparent to fishery workers. The immediate need for such a unit in Newfoundland was for collecting live salmon parr for laboratory experiments on tagging. The intention was to assemble a unit similar to one described by Haskell *et al.* (1954). Difficulty was experienced in obtaining the specified parts locally, but this was overcome by obtaining a packaged D.C. unit, the Vibrapack, with a similar circuit from P. R. Mallory & Co. Inc., Indianapolis, Indiana.

Description

The complete shocker is shown in Figure 1. It consists of a 6-volt motorcycle battery, the Vibrapack, and a voltmeter housed in a fairly water-tight box and two paddle-type electrodes. The outside dimensions of the box are 15 inches wide, 15 inches high, and 8½ inches deep. A pack-sack frame is attached to the box so that the outfit can be carried comfortably on the back. The electrodes, which are made of ½ inch copper tubing, are 4½ feet long, the round end being 7 inches in diameter. The grid in the round end is formed of heavy copper wire. A switch in the low voltage D.C. circuit for starting and stopping the unit is conveniently located on the handle of the right or positive electrode. A switch for use as a main switch or in emergencies is situated on top of the case. It has a pull cord which is readily accessible to the operator or the second man of the team. The unit SHOULD NEVER be used by one man alone.

The total weight of the outfit is 44 pounds, so it can be carried fairly easily by one man. The cost in St. John's, Newfoundland, was $120.00. (It should be less in most other cities in Canada.) The following is a list of parts and materials used: —

Parts

Battery	6-volt motorcycle battery, Lucas PUZ 7E-9
Vibrapack	Mallory VP-555H
Voltmeter	0-500 V.D.C., 1,000 ohms per volt, Triplett
Main fuse	30 amp.
Vibrapack fuses	10 amp. 3 AG
Switch No. 1	Open knife, SPDT, mounted on outside of case

Switch No. 2.....Slide switch, SPST, mounted on positive electrode handle

Relay.....Automobile horn relay, Sorensen HR-2

Discharge resistor R-4.....400 K safety resistor

Materials

Electrodes.....$\frac{1}{2}$ inch copper tubing

Electrode handle insulation.....$\frac{5}{8}$ inch automobile heater hose

Electrode connections.....For positive electrode, three conductor No. 12 cab tyre

For negative electrode, single conductor No. 12 cab tyre

Battery connections.....No. 6 insulated flexible cable

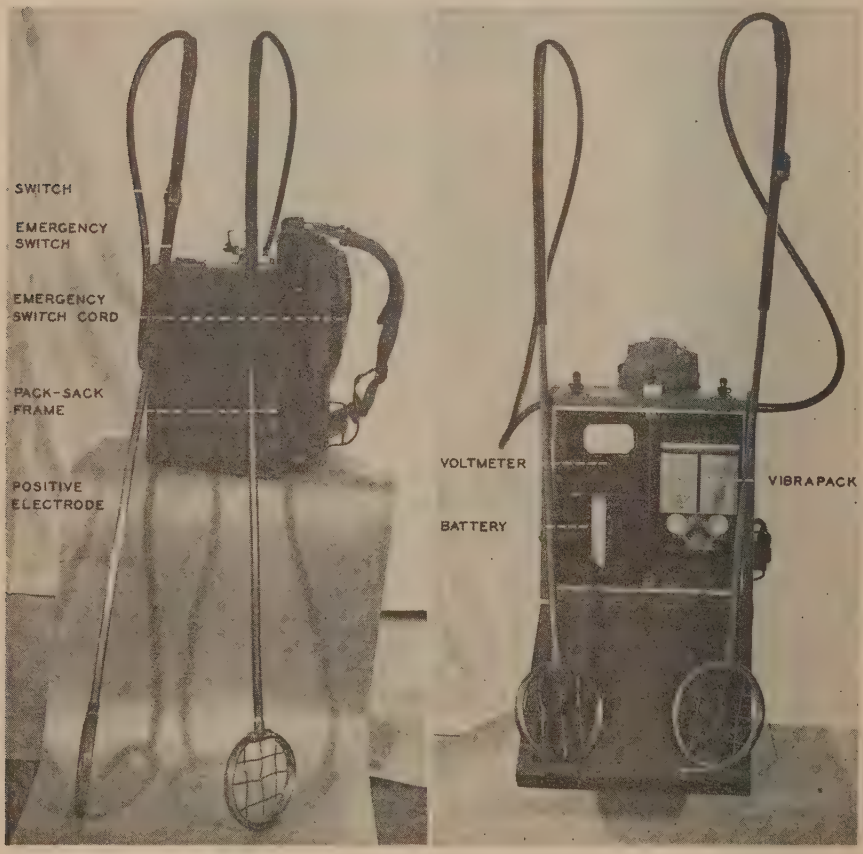

Figure 1.—On the left a view of the shocker ready for use, and on the right a view of the interior.

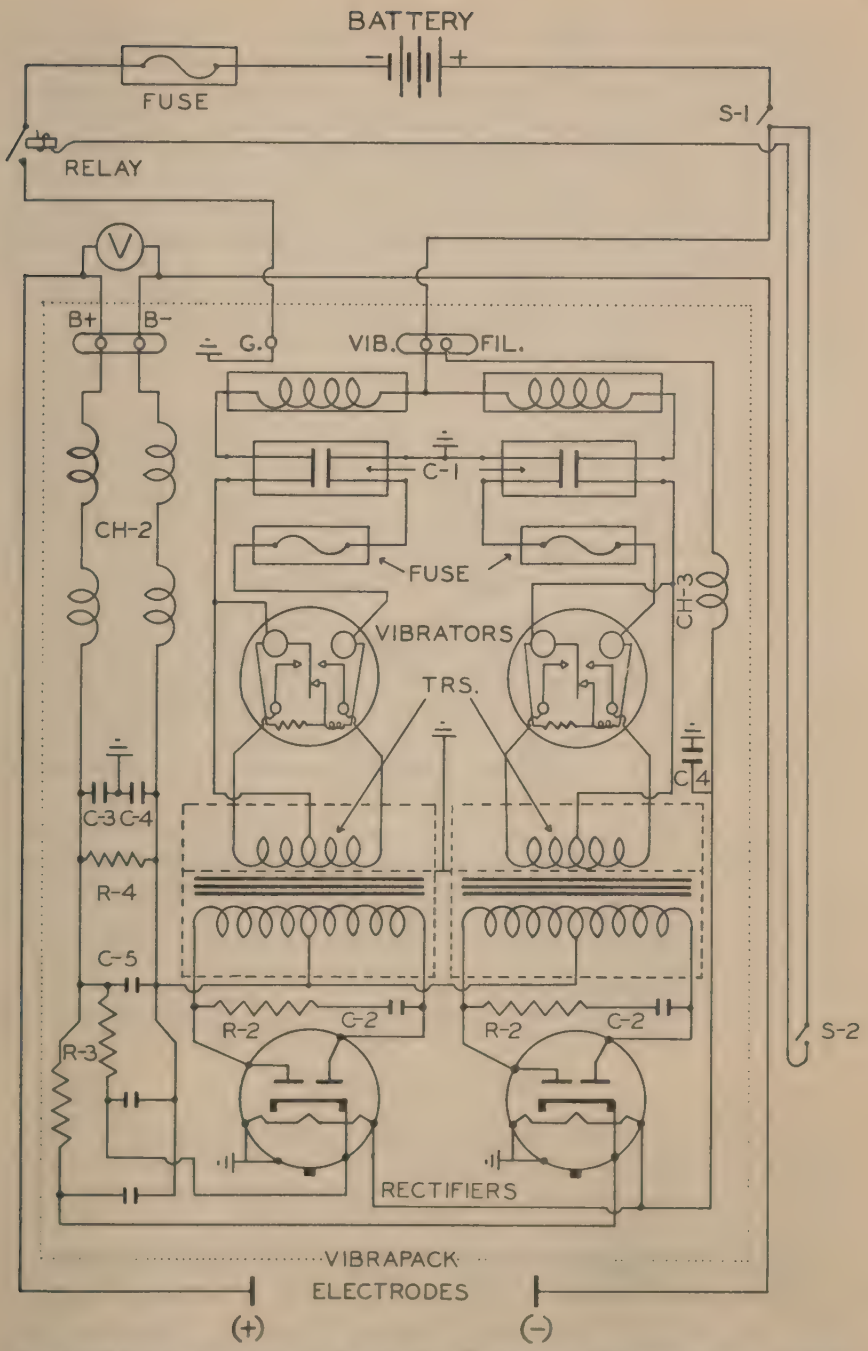

Figure 2.—Schematic wiring diagram. Symbols: B−, battery negative, B+, battery positive, C-1, C-2, C-3, C-4, condensers, CH-2, CH-3, chokes, FIL., filament, G, ground, R-2, R-3, R-4, resistors, S-1, S-2 switches; TRS., transformers; VIB., Vibrapack; and Ⓥ, voltmeter.

The Vibrapack or main power unit is factory built. Types are available for nominal input of 6, 12, and 32 volts, nominal output ranging from 125 to 400 volts, and maximum output of 60, 100, 150, and 200 milliamperes. The one selected for operation in Newfoundland streams has a nominal input of 6 volts, a nominal output of 300 volts, and a maximum output of 200 milliamperes. The wiring diagram of the Vibrapack unit is shown within dotted lines in the schematic wiring diagram of the shocker (Fig. 2). For ease in assembly and to give good connections all wires have soldered terminals. The Vibrapack is supplied with OZ4A rectifier tubes which according to the manufacturer's description require a minimum current load of 140 milliamperes to start the unit. However, in this particular installation it was found that the unit would start with a load as low as 90 milliamperes.

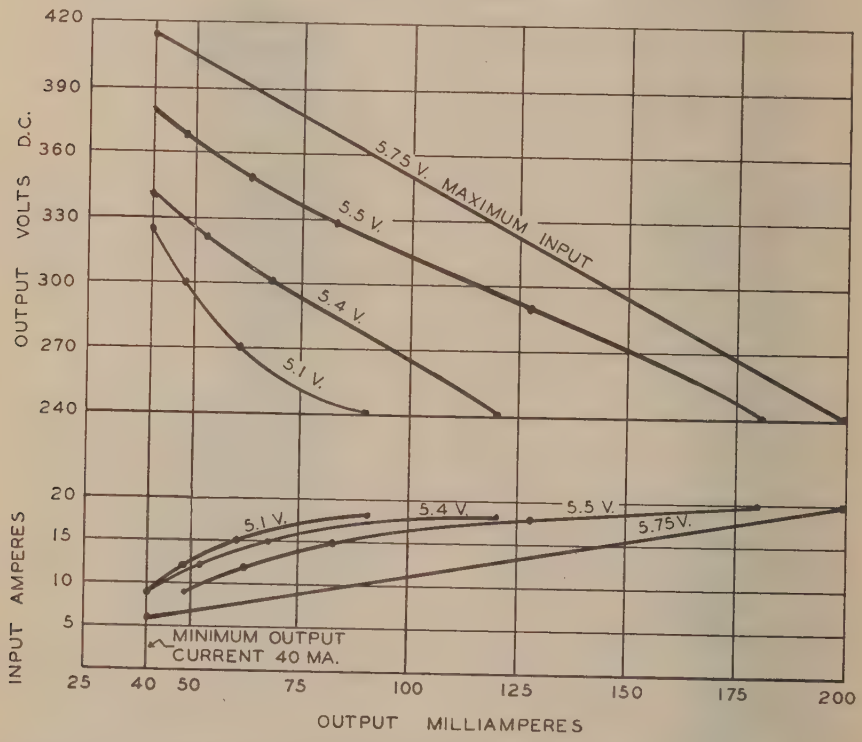

Figure 3.—Operating characteristics of the shocker in 6 inches of water with distances of 6 inches to 4 feet between electrodes.

Operation

The power to operate the shocker is supplied by a 6-volt motorcycle battery. This direct current is changed successively as follows: to low voltage alternating current by a vibrator, to high voltage alternating current by a transformer, and to high voltage direct current by a rectifier. This high voltage direct current is conducted to the electrodes for shocking the fish. The operating characteristics of the shocker

(Fig. 3) were determined in 6 inches of water in a wooden trough, 4 feet long and 14 inches wide, with distances of 6 inches to 4 feet between electrodes. All readings depend on electrode separation and immersion. The drain on the battery ranged from 5.1 to 5.75 volts and from 6 to 20 amperes. The output of the unit ranged from 240 to 415 volts and from 40 to 200 milliamperes. When the battery cannot produce 18 amperes at 5.1 volts, the unit will not start. If the output drops below 40 milliamperes at 240 volts, the unit will cease to operate. If the electrodes should accidentally come closer together than 6 inches at any time, a blown fuse will result, safeguarding the unit from overloading and damage to the component parts.

The shocker has worked quite well in the streams fished so far where depths ranged from 6 inches to 3 feet. Fishing downstream was found to be more effective than fishing upstream. A team of two men is required, one man to operate the shocker and the second man to do the collecting. In clear water with little current, the collector used a dip net and moved along with the operator. In turbid or swift water, the collector used a one-man seine and took up a stationary position while the operator started about 20 feet above and worked down to the seine. Fish were narcotized within 1 foot of the positive electrode. Beyond that, some were attracted to the positive electrode but most appeared to receive a slight shock and moved in all directions. Occasionally fish were led into the seine. All fish recovered quickly.

Since the unit has been quite effective in occasional collections, it shows that a current of 40 to 200 milliamperes is ample for small stream sampling in soft waters. The unit has not been used in hard waters, which would require a higher output current (Haskell *et al.*, 1954). Two fully charged batteries have supplied enough power for a full day's fishing. One battery will operate the unit continuously for 1 hour. Under actual fishing conditions where the unit is not in continuous operation, it is estimated that the battery will operate the unit effectively for a maximum of 2 hours. Fishing time is thus limited by the number of batteries available or distance travelled from a charger. A word of caution is necessary in connection with shutting off the unit. This should be done while the electrodes are immersed in the water so as to drain off any current left in the condensers. Otherwise the resultant shock upon contact with any of the uninsulated parts is quite uncomfortable and may cause other accidents. This danger could be eliminated by inserting a discharge resistor of 400 K across the output leads. It is shown as R-4 in the schematic wiring diagram (Fig. 2).

Acknowledgements

The fish shocker described in this paper was assembled by Mr. A. P. Cowan who is a technician on the staff of the Fisheries Research Board of Canada Biological Station at St. John's, Newfoundland. He also prepared the figures. Mr. W. M. Gaskell, Electrical Equipment Technician with the Newfoundland Light and Power Company, Limited, very kindly checked the technical parts of the paper.

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The Queen's Printer and Controller of Stationery, Ottawa, 1959

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Toxicity of a DDT Forest Spray to Young Salmon

by

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In June, 1957 an aerial DDT spray programme was carried out to control black-headed budworm in an area of northern Vancouver Island, B.C. This report summarizes a series of laboratory tests conducted to determine the tolerance of a representative species of Pacific salmon to the DDT formulation used in the spray programme. The study provides information relating to an extensive field survey conducted to assess the effects of spray deposition on aquatic fauna within the sprayed area (Crouter and Vernon, 1959, see page 23, this issue).

Method

Following consultation with members of the Department of Fisheries and B.C. Game Commission, the following programme was undertaken:

(*a*) Examination of tolerance to Atlox 2082A, the spray formulation emulsifier.

(*b*) Examination of tolerance to the spray formulation used in the aerial spray programme.

(*c*) Comparison of the potency of the aerial spray formulation with a DDT-acetone standard.

Accordingly, the following materials were used in the tolerance tests:

(*a*) Emulsifier —Atlox 2082A, a preparation of "alkyl aryl sulphonate" and "polyoxyethylene sorbitan esters of mixed fatty and resin acids" manufactured by the Atlas Powder Company.

(*b*) Aerial Spray Formulation —made up in the following manner:

25 g. 100% Tech. "Montrose" DDT
7.14 g. Base Oil
3.6 g. Atlox 2082A

One volume of the above mixture is diluted with 1.2 volumes of medium grade diesel oil. The "base oil" used was "Standard Base Oil (Wood Treating)" manufactured by the Standard Oil Company. "Medium grade diesel oil" was obtained from the Imperial Oil Company of Canada. DDT was obtained from the Stauffer Chemical Company, Portland, Oregon.

(*c*) DDT-Acetone Standard —Maintaining the same proportions of DDT and Atlox 2082A as in the aerial spray formulation, acetone was substituted for the base oil and medium grade diesel oil.

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Estimation of DDT content of solutions used in the tolerance tests and of the water samples collected in the spray area was carried out by a modification of the method suggested by Amsden and Walbridge (1954). A standard curve was prepared by analyzing for known amounts of DDT in acetone solution. No significant difference was obtained in a comparison of results from these standards with those obtained using aerial spray formulation added to water with subsequent separation of DDT with isopropyl ether. The resulting standard curve relating DDT concentration to spectrophotometric absorbance was used for estimating DDT levels in the succeeding tests. In all cases absorbance corrections were made with reagent blanks run concurrently. The method provided measurement of DDT down to 0.01 p.p.m. after extraction of the DDT from water. Colorimetric determinations were made using a Beckman DU Spectrophotometer at a wave length of 4700 A° and 0.03 mm. slit width.

Coho salmon (*Oncorhynchus kisutch*) underyearlings were considered as an appropriate species for determination of tolerance to DDT. The tests conducted with Atlox 2082A were carried out in August, 1957, and the coho underyearlings used averaged 6.89 cm. in length and 4.08 g. in weight. Test fish were exposed to various concentrations of Atlox 2082A at a ratio of 0.88 g. of fish per litre of solution. The tests conducted with the aerial spray solution and DDT-acetone standard were carried out in October, 1957. The coho underyearlings used averaged 8.81 cm. in length and 8.20 g. in weight. Test fish were exposed to various concentrations of the two materials at a ratio of 1.8 g. of fish per litre of solution. Fish used in the experiments were acclimated to and tested at 10°C.

One-half (23 litres) of the total volume of each test container was exchanged daily to minimize the possible diminution in DDT content by loss to the test fish. Daily determinations of DDT content were made by withdrawing one litre of each test solution and extracting the DDT with isopropyl ether. Such samples were taken at the beginning of each experiment, and immediately before each 23 litre solution exchange.

Results

Toxicity of Atlox 2082A to coho salmon underyearlings

Samples of 10 fish were exposed to each of eight concentration levels of Atlox 2082A from 500 p.p.m. to 10 p.p.m., along with a control. Times to 50 per cent

Table I

Time to 50 per cent sample mortality (ET_{50}) for samples of 10 coho underyearlings exposed to various concentrations of Atlox 2082A in fresh water.

Concentration of Atlox 2082A, p.p.m.	ET_{50}, minutes
500	33
100	98
70	290
50	380
40	680
30	1,125
20	2,450
10	>12,517
Control	No mortality

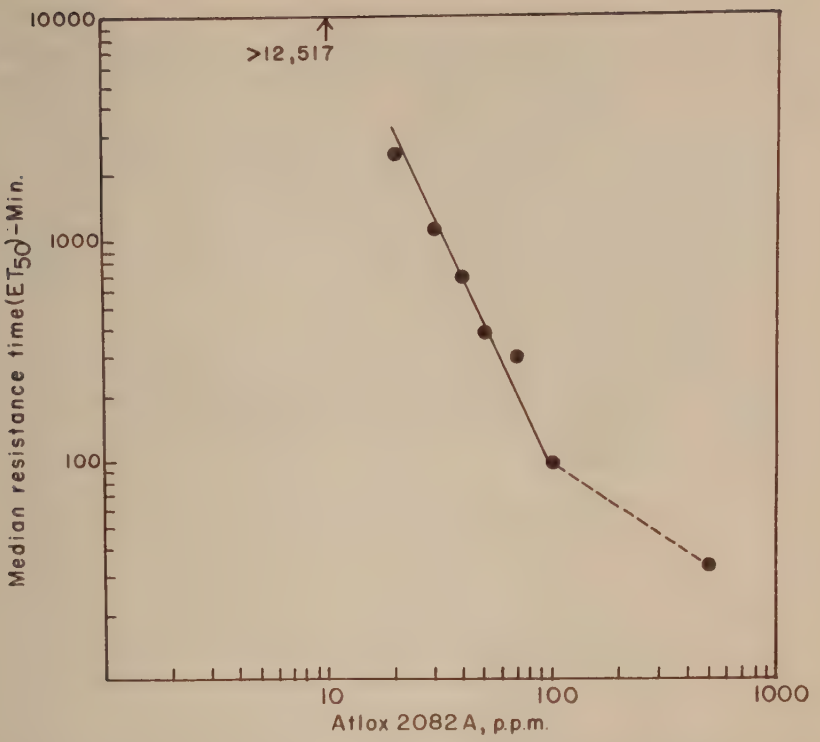

FIGURE 1—*Times to 50 per cent mortality in samples of 10 underyearling coho exposed to various concentrations of Atlox 2082A. The arrow in the upper corner refers to a test at 10 p.p.m. which was discontinued at 12,517 minutes.*

mortality in each concentration were calculated from the distribution of mortality times. Results of the tests are listed in Table I and illustrated in Figure 1.

Analysis of the transformed data provides an equation for the line from 20 to 70 p.p.m. From the equation the 48 hr. TL_m (median tolerance limit) (see Doudoroff, et al 1951) may be inferred and is estimated as 20.7 p.p.m. of Atlox 2082A. A safe level of Atlox 2082A, in terms of the experimental procedure, is estimated as 0.1 x 48 hr. TL_m or 2.1 p.p.m. Atlox 2082A. On the basis of the aerial spray formulation a unit of the spray in water containing 1 p.p.m. of DDT would contain about 0.15 p.p.m. of Atlox 2082A. Therefore, the concentration of DDT in a unit of water containing 2.1 p.p.m. Atlox 2082A would be approximately 14.0 p.p.m. DDT.

Toxicity of Aerial Spray Formulation to Coho Salmon Underyearlings

Samples of 10 fish were exposed to each of six concentration levels of the aerial spray formulation in water. The formulation does not form a stable emulsion in water and partition of the oil and aqueous layers occurred very rapidly. It was assumed for this reason that only a limited amount of the theoretical doses of DDT applied would be available throughout the water mass. In the higher concentrations tested a film of oil was visible on the water surface of the test containers. Subsequent tests also

indicated a considerable amount of the DDT in the test tanks was being adsorbed upon sediment in the freshwater supply and precipitated with that sediment to the bottom of the tanks. The latter fact has been well documented in the literature (see Berck, 1953). For these reasons the remaining concentrations established for tolerance tests are reported both as theoretical doses (level of DDT concentration by volume dilution) and as actual doses (level of DDT by chemical analysis of water taken midway between the surface and bottom of the test containers). Results of the tests on the aerial spray formulation are listed in Table II. Probit response curves for the concentrations examined are illustrated in Figure 2. The ET_{50} values derived from these curves are illustrated in Figure 3.

The median resistance times describe a most unusual response distribution. Maximum response in terms of time to death occurs at a dilution of the spray formulation providing a theoretical dosage of 0.5 p.p.m. (actual dosage of 0.05 p.p.m.). Test fish were less susceptible to the solutions at dosages both less and greater than 0.05 p.p.m. DDT (actual dosage).

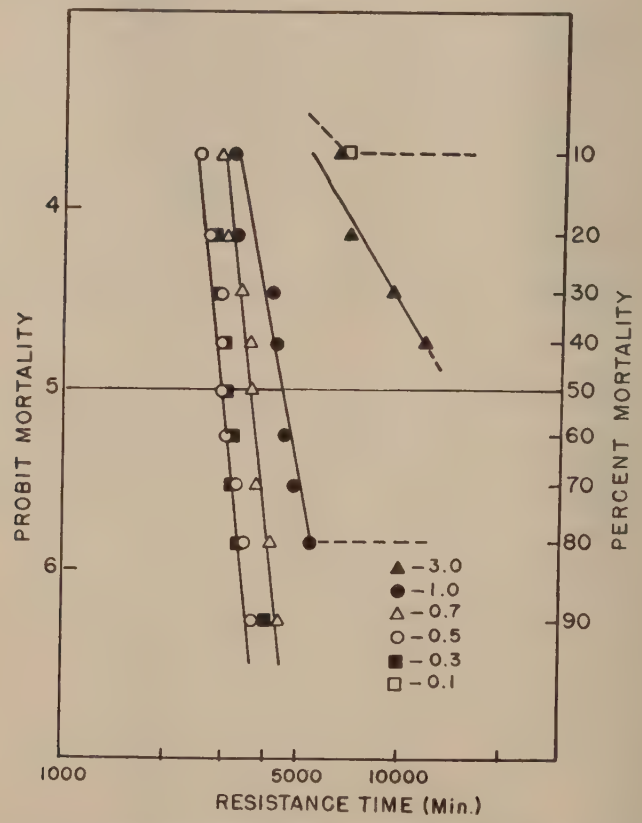

FIGURE 2—*Probit response curves illustrating the rates of mortality of coho underyearlings exposed to various concentrations of the aerial spray formulation. The curves are identified as to the theoretical concentrations of DDT applied, in p.p.m.*

Table II

Time to 50 per cent mortality (ET_{50}) for samples of 10 coho salmon underyearlings exposed to various concentrations of the aerial spray formulation.

Dilutions of aerial spray formulation providing DDT at the following theoretical concentrations, p.p.m.	Average concentration of DDT by chemical analysis, p.p.m.	ET_{50}, minutes
3.0	0.36	>12,492
1.0	0.19	4,400
0.7	0.11	3,600
0.5	0.05	2,950
0.3	0.10	2,950
0.1	0.07	>14,459
0.03	0.02	>10,031
Control	Control	No mortality

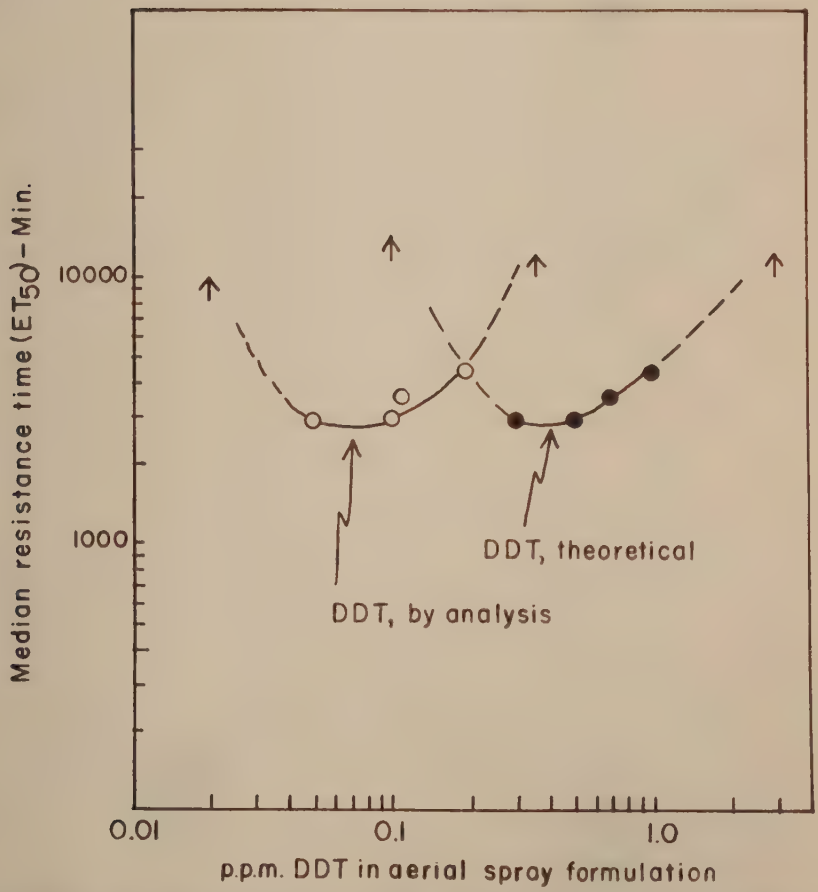

FIGURE 3—*Times to 50 per cent mortality in samples of 10 underyearling coho exposed to various concentrations of the aerial spray formulation. The arrows refer to tests in which 50 per cent mortality had not occurred at the times when the tests were discontinued.*

A further observation made in the 3.0 p.p.m. theoretical concentration (0.36 p.p.m. by analysis) supports field evidence of the Department of Fisheries (private communication). After 11,000 minutes of exposure almost all fish still living showed symptoms of blindness. Quite evident was the opacity of the lens of one or both eyes of these remaining fish.

Toxicity of DDT-Acetone Standard

Samples of 10 fish were exposed to each of six concentrations of a DDT-acetone standard. Although a highly stable emulsion was produced by the mixture of these materials, there was a tendency for the DDT to crystallize out onto the surface of the water solution, apparently on evaporation of the acetone carrier. Again it was suspected that DDT would be adsorbed onto particulate matter in the water, with a resulting diminution in the amount of DDT available throughout the water mass. Results of this series of tests are listed in Table III. The distribution of median resistance times is illustrated in Figure 4.

Table III

Time to 50 per cent mortality (ET_{50}) for samples of coho salmon underyearlings exposed to various concentrations of the DDT-acetone standard.

Dilutions of the reference solution providing DDT at the following theoretical concentrations p.p.m.	Average concentration of DDT by chemical analysis, p.p.m.	ET_{50}, minutes
3.0	0.31	850
1.0	0.29	730
0.7	0.06	850
0.5	0.05	1,100
0.3	0.09	1,150
0.1	0.08	1,750
0.07	0.05	>10,075
Control	Control	No mortality

In this series nearly all test fish died within the first 48 hours, consequently few analyses were possible for actual DDT content of each test solution. Some of the error in the response distribution for this series is undoubtedly connected with inability to sample at shorter intervals. A further source of error would be associated with variations in the amount of particulate matter in the test solutions.

Analysis of the transformed data indicates considerable variation in the distribution of resistance times; nevertheless, the trend in median resistance times is considered to be linear (Figure 3). Abrupt changes in slope within the probit response curves from 0.1 to 0.7 p.p.m. DDT theoretical dosage suggested that the response of the fish to the solutions might have been changing with evaporation of the acetone carrier.

Interpretation of Results

A limiting concentration of Atlox 2082A is estimated to be approximately 2.1 p.p.m. and, proportionately, this amount would be found at a DDT level of 14.0 p.p.m. The action of Atlox 2082A as a toxic agent would probably be found only at

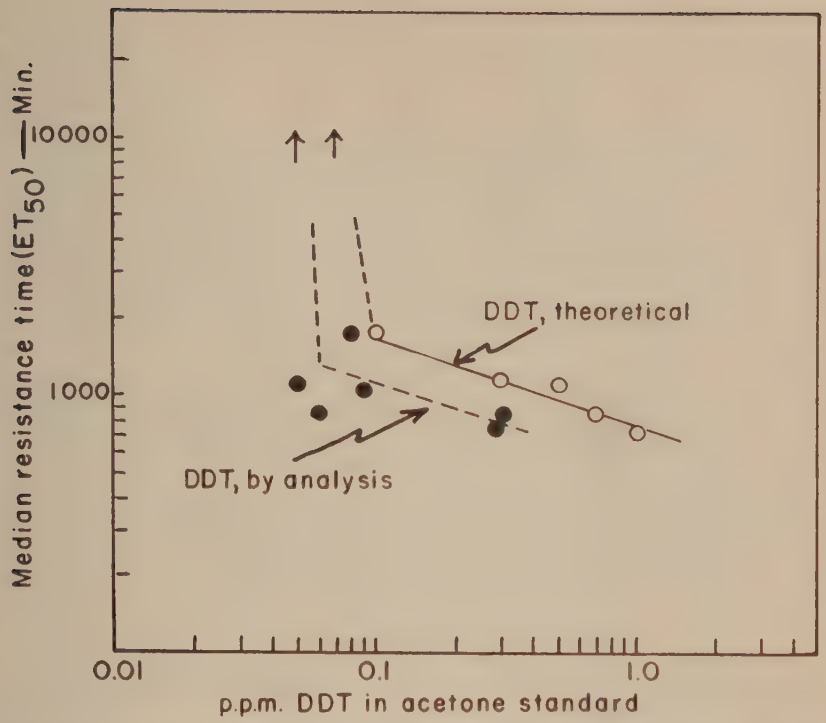

FIGURE 4 *Times to 50 per cent mortality in samples of 10 underyearling coho exposed to various concentrations of the DDT-acetone standard. For an explanation of the arrows, see Fig. 3.*

extremely high concentrations of spray formulation. It is doubtful if such concentrations would be found under spray conditions.

No valid statistical comparison can be made between the response distributions for the aerial spray formulation and DDT-acetone standard as that of the former is not linear. The bizarre distribution of resistance times for the aerial spray formulation suggests that the physical aspects of contact with the spray formulation or its components are dissimilar within the range of concentrations examined. In terms of DDT available in the water mass, the aerial spray caused mortality at and above 0.05 p.p.m. whereas 0.02 p.p.m. available DDT did not affect the test fish within the experimental period. In the case of the DDT-acetone standard, the lowest concentration producing mortality was again 0.05 p.p.m. available DDT. However, as the 0.07 p.p.m. test (0.05 p.p.m. available DDT) did not cause mortality within the experimental period, whereas the 0.5 p.p.m. (0.05 p.p.m. available DDT) did, factors may operate to determine toxicity other than that of the final amount of DDT available. Comparing the two response distributions from another aspect, the resistance times of the samples of young salmon exposed to the aerial spray are greater than those for the fish exposed to the same range of DDT concentrations in the DDT-acetone standard, even though this range ultimately was lethal in both cases.

In summary, and in terms of the test conditions, the aerial spray formulation may be regarded as "safe" to young coho salmon below approximately 0.05 p.p.m. where the measure of safety is regarded as the absence of mortality within an exposure period of one week at 10°C. An unusual response distribution was found for the aerial spray with resistance times increasing with increase in dosage over the higher portion of the concentration range examined. Associated with high concentration of spray, however, was development of blindness in the test fish. The series of tests tended to point up the complexity of the problem of interpretation of the action of associated variables. Besides the measure of DDT available to the test fish within the water mass, it is considered that the stability of the solution in such aspects as volatility, gravity stability, adsorptive potential of inclusions in the water supply and action of associated formulation components may have a considerable influence on the biological activity of the formulation used. Indeed, such factors may to a large extent be responsible for some of the diversity of tolerance estimates reported in the literature. A thorough study of the relation of such physical characteristics to biological effects on fishes would be a useful adjunct to such tolerance studies.

It should also be recognized that the criterion of safety applied here for young salmon does not necessarily relate to the impact on the total biological community. The tests do indicate, however, the range of concentrations of DDT and the related exposure times which could be harmful in the field.

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The Queen's Printer and Controller of Stationery, Ottawa, 1959

THE CHEMICAL COMPOSITION OF THE GASTRIC SHIELD OF THE OYSTER CRASSOSTREA VIRGINICA (GMELIN)

Barbara L. Shaw and Helen I. Battle

The gastric shield of *Crassostrea virginica* is a bilobed laminated structure composed of an opalescent translucent material superficially resembling the matrix of hyaline cartilage, although exhibiting an acidophilic rather than a basophilic reaction to stains. Shaw and Battle (10) were in agreement with Nelson (8) in describing it as "chondroid-like". Berkeley (1), on the contrary, demonstrated the chitinous nature of the gastric shield of the lamellibranch *Schizothoerus nutalli* and concluded that it is lacking in any chondrin-like constituent.

Structurally the molluscan gastric shield in certain respects may be analogous to the peritrophic membrane of insects, which Wigglesworth (11) has shown, in some instances, to be composed of concentric lamellae secreted by the underlying epithelial cells of the mid-gut. It has been demonstrated that the gastric shields of a number of molluscs are resultant from a fusion of droplets of secretion from underlying epithelial cells, viz.: *Mytilus* (List (7)), *Anodonta* (Gutheil (4)), *Ostrea chilensis* (Dahmen, (3)), *Ostrea angulata* (Leenhardt, (6)). Yonge (12), however, has supported the viewpoint that the shield in *Ostrea edulis* is not a secretion but is formed by fusion of cilia in response to the irritation caused by the head of the style. In *C. virginica*, cytoplasmic strands are evident between the gastric shield and the underlying gastric epithelium. Hence, if the shield represents a product of epithelial cells, rather than being comparable to an amorphous connective tissue-ground substance, as inferred in the description "chondroid-like", the statement of Shaw and Battle (10) merits re-examination. Accordingly shields of this species have been subjected to various chemical tests for chitin.

Richards (9) has shown that pure chitin, like many other substances, is colored brown by iodine but chitosan in an acidified medium takes on a violet or a reddish-violet coloration. Since very few organic substances will withstand the alkaline treatment necessary to convert chitin to chitosan, he considers that this iodine–chitosan color test is completely valid when positive. Gastric shields of *C. virginica*[1] were dissected entirely free from adhering gastric epithelial tissues and treated according to the method of Campbell (2): small test tubes containing shields in 5 ml of KOH saturated at room temperature were closed with a Bunsen valve and slowly heated in a glycerine bath to 160° C for 15 minutes. On microscopic examination after they were cooled to room temperature, shields invariably appeared quite intact and of characteristic shape. They were transferred to water and dehydrated by passage through a graded series of alcohols prior to drying.

[1]Supplied by Fisheries Research Board of Canada.

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The following characteristic reactions for chitin transformed to chitosan were positive in each instance:

(*i*) Slow solution in 3% acetic acid and appearance of a white precipitate on addition of 1% sulphuric acid.

(*ii*) Brown coloration appeared rapidly in 0.5% iodine in potassium iodide. Following removal of excess iodine solution and replacement by 1% sulphuric acid, the edges of the shields became reddish violet and the central thicker area almost blackish. On replacement of the dilute acid by 75% sulphuric acid, the color gradually faded to yellow at the edges and the whole shield ultimately became colorless.

In addition, shields stained a dark brownish black in pyrogallic acid after they were boiled in 60% potassium hydroxide, indicating a positive reaction for chitin according to the method of Kennedy (5).

On the bases of the above tests, there would seem to be positive evidence that the gastric shield of *C. virginica*, like that of *Schizothoerus nutalli* (Berkeley (1)), is chitinous rather than chondroid in nature.

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Effects of Spruce Budworm Control on Salmon and Other Fishes in New Brunswick

by

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Each spring since 1952 large forest areas of northern New Brunswick have been sprayed with DDT in efforts to control a spruce budworm epidemic. The DDT has been mixed with a special solvent oil and sprayed from airplanes at a concentration of one half pound per acre (one pound per acre in 1952). Some areas have been resprayed at intervals of one, two or three years. In spite of these extensive and costly control measures, the budworm population continued to expand until in 1957 over five million acres of New Brunswick woodland was sprayed, much of it for the second or third time.

In the face of such widespread and repeated applications of DDT many biologists, sportsmen and naturalists have become increasingly worried about the side effects of the insecticide on fish and wildlife in the budworm-infested areas. Fisheries workers have been especially concerned because many species of fish are known to be extremely sensitive to DDT.

Fortunately, a unique opportunity has arisen to study the effects of this DDT spraying on the fish populations of some New Brunswick rivers. Since 1950 the Fisheries Research Board of Canada, through its Biological Station at St. Andrews, has carried out an annual fall census of the young salmon in several branches of the Miramichi River system of central New Brunswick. This programme has involved sampling the fish populations by seining with electrofishing at the same localities each year. Censusing began on the Northwest Miramichi River in 1950, was extended to the Dungarvon River in 1952 and to the Renous and Cains Rivers in 1955. In 1957 the work on the Dungarvon River was discontinued to enable censusing to begin on the Tobique River, the most important salmon angling tributary of the St. John River. A total of 34 census stations is involved: ten on the Northwest Miramichi, six on the Dungarvon, five on the Cains, three on the Renous and ten on the Tobique.

Effects of DDT spraying on young salmon

The relative abundance of young salmon in the Northwest Miramichi branch of the Miramichi River each year from 1950 to 1957 is shown in Figure 1. Data from the first year of censusing (1957) on the Tobique River are included in the figure for comparison. All data are expressed as numbers of fish per 100 square yards of river bottom. The fish are separated into three groups on the basis of size. The smallest fish are fry or underyearlings. Parr less than 10 cm. total length are classed as "small parr"; those over 10 cm. as "large parr". In general, these three size groups correspond to the first three years of life. In the Miramichi River system most young salmon go to sea as smolts at the beginning of their fourth summer.

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Populations of all three size groups of young salmon in the Northwest Miramichi increased gradually from 1950 to 1953 (Figure 1). This increase is probably related to the experimental merganser control which has been carried out on the river since 1950. Control of American mergansers on the Pollett River, N.B., between 1947 and 1950 resulted in a marked increase in the number of smolts leaving the river (Elson, 1950). Following this evidence that reduction of merganser populations benefited young salmon, merganser control was extended to the Northwest Miramichi on an experimental basis in 1950. The experiment was designed to determine whether control of mergansers would eventually increase the numbers of adult salmon from this river taken in the sport and commercial fisheries.

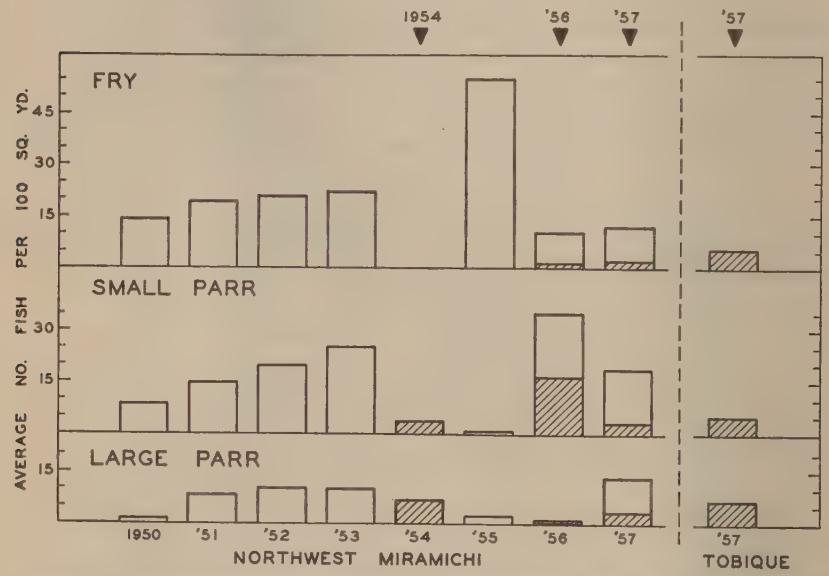

FIGURE 1. *Abundance of young salmon in the Northwest Miramichi and Tobique Rivers. Solid triangles indicate years when DDT was sprayed in the area. Cross-hatching indicates fish found in sprayed parts of rivers.*

In 1954 all stations sampled on the Northwest Miramichi were within the area sprayed with DDT for the first time. The drastic effects of the spray are clearly seen in Figure 1. Not one fry was found that year. Small and large parr were also reduced but to a lesser extent than the fry. Direct evidence of the harmful effects of the DDT was obtained in 1954 by holding salmon parr in cages in several parts of the Northwest Miramichi. From 63 per cent to 91 per cent of those held within the spray area were dead in three weeks, while only 2 per cent died in an unsprayed control stream during the same period (Kerswill and Elson, 1955). The extreme sensitivity of young salmon to DDT was also demonstrated by laboratory bio-assays carried out in the summer of 1957. The *median tolerance limit* (concentration killing one half a sample of fish) for small parr in DDT was 0.049 ppm. for a 24-hour period, and 0.047 ppm. for 48 hours (Keenleyside, —1958).

No spraying was done in the Northwest Miramichi region in 1955 and fry were unusually abundant that year (Figure 1). This heavy fry population may have been due to several factors: (1) less competition for food and space with salmon parr, many of which were killed by the 1954 spraying; (2) great abundance of aquatic Diptera larvae which, in contrast to many of the larger aquatic insect larvae, survive the budworm spraying and which form an important part of the diet of salmon fry in this area; (3) unusually successful spawning of adult salmon in the Miramichi area in 1954.

Small and large parr were scarce in 1955, due to the lethal effect of the 1954 spraying. Those small parr found in 1955 may have been fry which were missed in the 1954 censusing, or they may have been small parr in 1954 which failed to reach large parr size by the fall of 1955 when censusing was done. The aquatic stages of many caddisflies, stoneflies and mayflies are greatly reduced by the DDT spraying (Ide, 1957). Since these insects form the major part of the diet of small and large salmon parr in the Miramichi area, a reduction in their numbers may be reflected in slower growth of the fish.

In 1956 and 1957 only the lower part of the Northwest Miramichi River was within the spray area. In both years approximately the same extent of the headwaters was free of spray. The bars on Figure 1 for 1956 and 1957 are partly cross-hatched and partly open, to represent the proportions of fish found in sprayed and unsprayed parts of the river respectively. Clearly the best fry survival occurred in the unsprayed headwaters. In 1956 small parr were abundant, even in the sprayed section of the river. These were survivors of the very large 1955 fry class. Many of them were in poor condition in the fall of 1956, due to the scarcity of large aquatic insects on which they usually feed. Small parr were surprisingly abundant in the river in 1957. Most of them were found in the unsprayed headwaters, where they were more numerous than the fry of 1956. Some of these fish were probably small parr the year before which had not reached large parr size by 1957. Large parr were scarce in the Northwest Miramichi in 1956 as a result of both the 1954 and 1956 sprayings. In 1957 they were numerous due to growth of the big 1955 fry class, some of which should have reached large parr size by 1957.

All 10 census stations on the Tobique River were within the area sprayed in 1957. Some of them were also sprayed in 1953, 1955 or 1956. Figure 1 shows that young Tobique salmon were found in moderate numbers only in 1957. The figure for fry is misleading, since all fry found in the river were taken at one station, where hatchery underyearlings were planted shortly before censusing. No fry were taken at the other nine stations.

All the data obtained from population assessments on four branches of the Miramichi River since 1950 are summarized in Table I. The table includes the Northwest Miramichi data of Figure 1, but not the Tobique data. Fluctuations in abundance of young salmon over this larger area have been similar to those on the Northwest Miramichi branch. In years when DDT was sprayed populations of young salmon in streams within sprayed areas have been reduced. This reduction has been greatest among the fry. One year later, with no further spraying in the same areas, fry have been very abundant, while small and large parr have been scarce. In areas where no respraying was done for two or three years fry populations were similar to pre spray levels. Parr, however, were more numerous than before, reflecting growth

Table I

Salmon abundance in the Miramichi River since 1950

	Number of stations studied	Average no. fish per 100 sq. yds.		
		Fry	Small parr	Large parr
Before Spraying	58	23.5	16.7	8.1
Same Stations After Spraying				
Year of spraying	34	2.5	9.4	3.7
No Further Spraying — 1 year later	17	50.8	0.6	2.8
No Further Spraying — 2 years later	6	24.2	43.9	2.6
No Further Spraying — 3 years later	3	27.8	48.4	29.7

and survival of fry of the first post-spray year. Also, slower growth of small and large parr, due to reduction of their food by spraying, probably results in some parr being included in the same size group for two consecutive years.

It should be emphasized that abundant post-spray populations of small and large parr have been found at only three census stations, where spraying has not been repeated for an interval of three years (bottom line of Table I). These three stations are in the headwaters of the Northwest Miramichi River, which is excellent parr-rearing water. Most of the Miramichi watershed within the range of the present spruce budworm outbreak has been sprayed on successive or alternate years since 1954. Since most young salmon in this area spend three years in fresh water before going to sea, respraying with DDT at intervals of less than three years can affect the same year-classes more than once and thus greatly reduce the potential salmon production of the affected rivers.

Effects of DDT spraying on other fishes

During the annual censusing of young salmon several other species of fish are regularly caught. Changes in abundance of these species have also occurred following DDT spraying. The relative abundance of brook trout (*Salvelinus fontinalis*), eels (*Anguilla rostrata*) and four species of "minnows" in the Northwest Miramichi River since 1953 is shown in Figure 2. Under "minnows" are included four species of Cyprinidae, the black-nosed dace (*Rhinichthys atratulus*), the common shiner (*Notropis cornutus*), the fallfish (*Semotilus corporalis*) and the chub (*Couesius plumbeus*).

Trout are found mainly in the headwaters of the Northwest Miramichi, a section of the river that was sprayed in 1954 only. They were scarce in 1954, recovered gradually until by 1956 they were more numerous than before spraying, and by 1957 were present in about average numbers again (Figure 2). Since the fishing gear used in censusing does not capture trout underyearlings efficiently, only data for yearling and older trout are included in the figure. Fluctuations among these age-groups are similar to those among salmon parr in this headwater section of the river. They are most abundant two years after spraying, when spraying is not repeated.

Eels are present in moderate numbers throughout the Northwest Miramichi. They appear to have been severely affected by the DDT spraying (Figure 2). Their numbers were reduced in 1954 and in 1956, and by 1957 none at all were found.

Minnows are found only in the lower part of the Northwest Miramichi, where spraying occurred in 1954, 1956 and 1957. They have remained abundant throughout this period and were particularly numerous in 1956 (Figure 2). The relative proportions of the four species in this river have been approximately the same during this period. Dace are about 10 times as abundant as each of the other species.

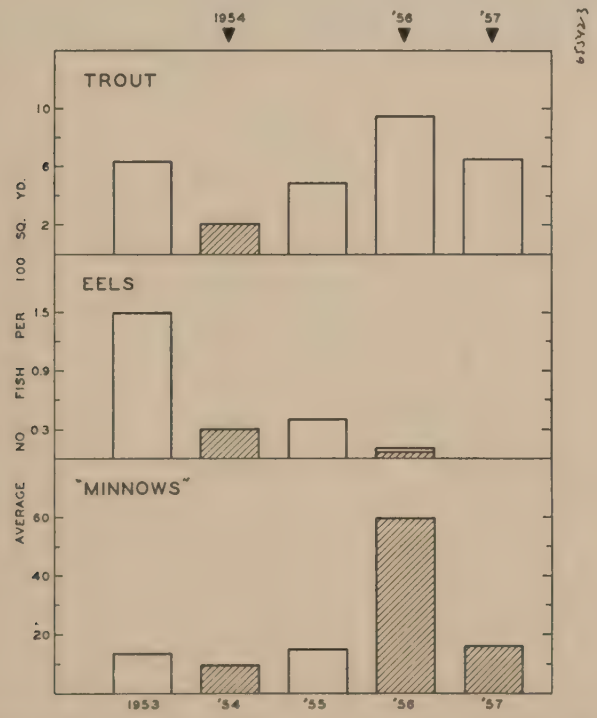

FIGURE 2. *Abundance of several species of fish in the Northwest Miramichi River. Symbols as on figure 1.*

Summary

Several species of fish in New Brunswick rivers have been adversely affected by the widespread aerial application of DDT to the forests of the province as a control agent for spruce budworm. Young salmon, brook trout and eels are reduced in number following spraying. Four species of cyprinids are relatively unaffected by the spray. Salmon populations appear to recover if spraying is not repeated over the same areas for an interval of at least three years. However, much of the budworm-infested woodland has been resprayed at intervals of less than three years. Under these conditions one age-class of salmon can be affected more than once and smolt production from rivers in the area will be seriously curtailed.

Acknowledgements

The raw field data collected annually by the census crews has been processed and tabulated by Dr. P. F. Elson. His assistance in the preparation of this paper is gratefully acknowledged.

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The Queen's Printer and Controller of Stationery, Ottawa, 1959

CALIPERIA BREVIPES N. SP. (CILIATA: PERITRICHA), EPIZOIC ON RAJA ERINACEA MITCHILL AT SAINT ANDREWS, NEW BRUNSWICK[1]

MARSHALL LAIRD[2]

Abstract

Two adults of the common skate, or tobacco box, *Raja erinacea* Mitchill, trawled in Passamaquoddy Bay, N. B., in June, 1958, bore myriads of peritrichous ciliates on and about the free edges of the gill filaments. The morphology of these epibionts refers them to the genus *Caliperia* Laird (Scyphidiidae), previously known from a single New Zealand species. *C. brevipes* differs from the genotype in having relatively shorter caudal processes, a posteriorly located micronucleus, and a prominent siderophilous collar at the cytostome. Comparisons are drawn between these organisms and *Ellobiophrya donacis* Chatton and Lwoff.

Introduction

Caliperia longipes Laird was described from the gills of two fishes of the intertidal zone collected near Wellington, New Zealand—a clingfish, *Oliverichtus melobesia* (Phillipps), and a blenny, *Ericentrus rubrus* (Hutton) (6). It is a peritrichous ciliate, characterized by an attachment organelle demarcated from the endoplasm of the zooid and having two caliper-like posterior processes. These contain a rod-shaped structure which runs the length of one of them, curves through 180° just short of the posterior limit of the body proper, and proceeds to the distal extremity of the other. They pass one on either side of a gill filament, grasping it firmly between them, thanks apparently to the elasticity of the recurved rod. Their free ends meet beyond the filament, and are pressed, but not locked, together.

There is close but superficial similarity between *C. longipes* and *Ellobiophrya donacis* Chatton and Lwoff, another peritrichous epibiont from the intertidal zone. *E. donacis* resembles the New Zealand ciliate in securing itself to the host (a European lamellibranch, *Donax vittatus* da Costa) by means of two posterior processes. These, however, contain no supporting organelle, their endoplasm is continuous with that of the zooid, and they interlock distally by means of a button-like structure (1, 2).

Until now, both genera have remained monospecific. Chatton and Lwoff (1) at first regarded their epibiont as the type of a new family, but subsequently (2) referred it to a new subfamily of the Vorticellidae, the Ellobiophryinae, allied to the Scyphidinae. Kahl (4) revised the order Peritricha, raising the Scyphidiidae to the same rank as the Vorticellidae (Peritricha: Sessilia) and transferring *Ellobiophrya* to the former family. Others (5, 6) have followed his lead, and *Caliperia*, too, is placed in the Scyphidiidae (6). It has been

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[2]Institute of Parasitology, McGill University.

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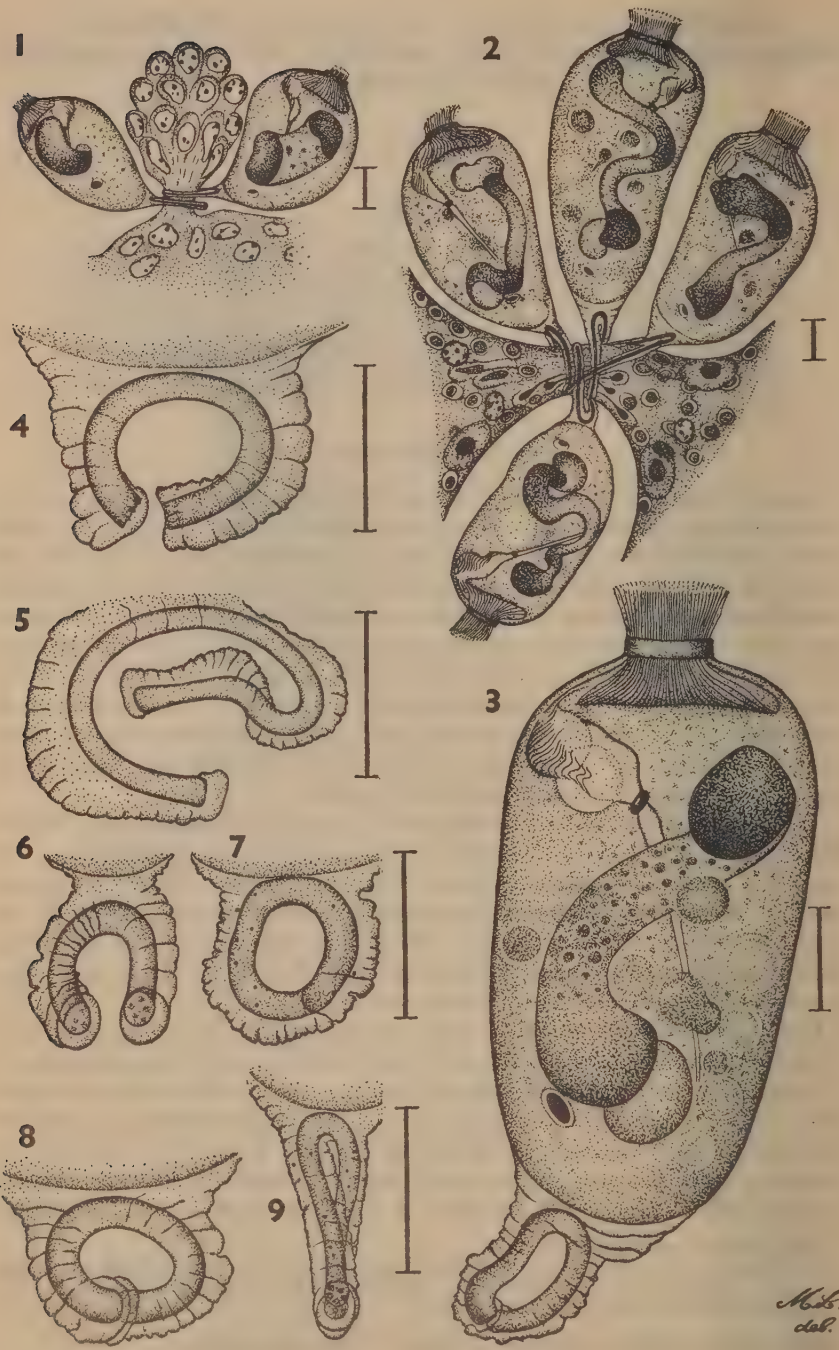
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suggested (6) that *Ellobiophrya* may be descended from an ancestor of the typical *Scyphidia* kind (in which the zooid itself is sharply constricted basally) and *Caliperia* from a *Gerda*-type scyphidian (having a more or less cylindrical zooid, the endoplasm of which is demarcated from a broadly based scopula). The button-like interlocking device of *Ellobiophrya* and the endoskeletal organelle of *Caliperia* are viewed as homologous with the scopular rodlets of the appropriate type of ancestral scyphidian.

A second species of *Caliperia* came to light in 1958 during studies of the parasites of marine animals undertaken at the Fisheries Research Board Biological Station, Saint Andrews, New Brunswick.

Methods and Material

Between June 17 and July 7, 1958, various marine and brackish-water organisms were brought to the laboratory alive and examined for parasites. These animals included 186 fishes of 25 species. As a routine measure, fresh saline preparations from the gills were covered with cover slips and examined by phase contrast. Very numerous examples of the ciliate forming the subject of this paper were discovered in slides from two adults of the common skate, or tobacco box, *Raja erinacea* Mitchill, trawled at 20 fathoms off Minister Island, adjacent to Saint Andrews, Passamaquoddy Bay, on June 19 and 24. All the other fishes, including an example of the starry or thorny skate, *Raja scabrata* Garman, proved negative for the organism, which is described hereunder. Permanent haematoxylin-stained mounts were made, as recently described in this journal (7). All dimensions are derived from the measurement of 50 consecutive examples, except where otherwise stated.

Caliperia brevipes n. sp. (Figs. 1–9)

Habit

Microscopic examination of pieces snipped from the gills revealed great numbers of peritrichous ciliates massed on and about the free edges of the filaments. In life these project into the cavity of a gill pouch, where their epibionts would be exposed to the full force of the branchial current. The ciliates were so tightly pressed together, the tapering posterior extremities of groups of up to 16 individuals being directed in towards a common center, that their real nature could not be determined *in situ*. Apart from the facts that they were relatively sluggish and did not expand the peristome, they bore considerable resemblance to *Epistylis* colonies.

FIGS. 1–9. *Caliperia brevipes* n. sp. All figures prepared with the aid of a Zeiss drawing apparatus, from haematoxylin-stained slides. The scale lines to the right of the figures represent 10 μ at the same magnification.

FIGS. 1 and 2. Habit. × 580.

FIG. 3. Whole animal, detached from host. × 1450.

FIGS. 4–9. The attachment organelle. × 2340.

When teased-up fragments of gill filaments are studied, though, it is seen that each zooid is quite independent of its neighbors and is secured in position by means of the distinctive attachment organelle that characterizes the genus *Caliperia*. The pseudo-colonial appearance of a heavy infestation is due to the frequent use of a single anchorage by two or more individuals. In some instances (Fig. 1) the point of attachment is the base of a bud-like outgrowth of the layer of cells forming the effective membrane across which gaseous exchange takes place in respiration (3), while in others (Fig. 2) it is one of the strands of the superficial capillary network. Elongation of the nuclei of epithelial cells near the attachment organelle (Fig. 1), and the greatly stretched dumbbell-shaped nuclei of erythrocytes in the most constricted parts of capillaries (Fig. 2), are evidence of the tightness of the grip of the ciliate's two caudal processes.

Morphology

Fresh examples examined in saline exhibit little activity. They are urn-shaped and widest anteriorly, the cilia protruding as a sheaf through an opening having a raised rim and corresponding to the neck of the urn. Phase contrast reveals the elongate macronucleus and numerous more or less spherical food inclusions. Under dry objectives the pellicle appears smooth (annuli were not searched for by oil immersion). Ten ciliates comprising a group which a piece of host tissue had protected from compression by the cover slip measured from 64.8 μ to 81.1 μ by 28.8 μ to 41.8 μ.

Examples in permanent mounts exhibit shrinkage due to fixation. They are also flattened during the smearing process, and this renders them relatively broader than in life. Otherwise, as in *C. longipes* (6), the contours of the zooid are little altered, since marked retraction does not take place. Forty (83.3%) of 48 extended ciliates were broadest anteriorly, while 18 (81.8%) of 22 slightly contracted ones were broadest medially. The posterior third was never broader than the remainder of the zooid. The body proper measures from 29.2 to 72.9 μ by 21.3 to 37.5 μ (av., 49.3 by 28.2 μ). Mounted zooids of *C. longipes* are of similar length but relatively broader (av., 51.5 by 38.8 μ). The caudal processes, ranging from 3.6 to 28.6 μ (av., 12.7 μ) in length by from 1.3 to 5.2 μ (av., 3.0 μ) in breadth, are much shorter and appreciably narrower than those of *C. longipes*, which measure from 37.5 to 155.6 μ (av., 90 μ) by 5 to 6 μ.

The siderophilous rim at the entrance to the peristome (Fig. 3) measures from 6.2 to 11.3 μ (av., 8.3 μ) in diameter. General details of the cilia, peristome, vestibule, and contractile vacuole are as in the genotype, from which the skate epibiont differs, however, in the more intense staining reaction of the walls of the vestibule and in having a prominent siderophilous sphincter (Fig. 3) at the cytostome. As in *C. longipes* the cytopharynx is a long tube discharging near the posterior part of the macronucleus. This structure is long and sinuous, measuring from 23.9 to 78.1 μ (av., 50.7 μ) in length and from 2.1 to 12.9 μ (av., 6.5 μ) in breadth. Like that of the genotype it exhibits

numerous chromatin spherules each surrounded by a clear halo. The oval to lenticular micronucleus measures some 4.5 by 2.4 μ and is rather larger than that of the New Zealand species, from which it differs radically in its posterior instead of anterior location.

Stretched to their fullest extent around a support, the caudal processes appear somewhat flattened and strap-like. When torn free during smearing, though, they are relaxed, superficially wrinkled, and round to oval in cross-section (Figs. 3–9). As is seen from the accompanying illustrations, the endoskeletal rod is always at the inner margin. This serves as an indication of the structure's elasticity, as does a figure-of-eight shape that the organelle sometimes assumes. Siderophilous granules may be present (Figs. 7, 9), but are less evident than in *C. longipes*. Closure of the attachment organelle is effected by inward pressure as in the latter species, the pellicle of the two processes sometimes being bulged and flattened at the point of contact (Fig. 8). Although the extremities of the endoskeletal rod appear somewhat rugose in favorably orientated specimens (Figs. 4, 6, 9), there is no locking device as in *Ellobiophrya*. Damage in smearing merely results in terminal contact being broken, each process remaining intact.

This epibiont from *Raja erinacea* is clearly referable to the genus *Caliperia*. Differing as it does from the only species previously described in its much shorter caudal processes, the posterior location of the micronucleus, the presence of a prominent siderophilous sphincter at the cytostome, and in other respects as detailed herein, it is designated *Caliperia brevipes* n. sp. in reference to the first and most obvious of these features. The type slide is in my collection.

Discussion

Caliperia brevipes resembles *Ellobiophrya donacis* in the relative length of the caudal processes and the presence of a siderophilous sphincter at the cytostome. Nevertheless, differences of at least generic significance still separate *Caliperia* from the lamellibranch epibiont. The latter is basally, not anteriorly, widest, and the endoplasm of the zooid is continuous with that of the caudal processes. These altogether lack an endoskeleton, but instead have a terminal locking organelle so efficient that under stress the attachment ring usually tears apart at some other point. Another feature peculiar to *E. donacis* is the elliptical instead of elongate shape of the macronucleus in its normal resting state (2).

E. donacis, as described and figured by Chatton and Lwoff (1, 2), hangs loosely, like a padlock, from a filament of the gill grid of the host. Its adaptations are excellently suited to this purpose, just as are those of the two species of *Caliperia* to attachment to the gills of fishes. All three ciliates obtain their food from the water streaming over the branchiae of the host. It is obviously advantageous for *Ellobiophrya* to be able to pivot about the anchorage to feed on either side of the gill grid and to conform to the lamellibranch's reversals of direction of the branchial current. Such reversals for

the purpose of clearing blockages occur in skates too from time to time (3), but a temporary change in the direction of flow does not necessitate any reorientation of a *Caliperia* projecting into the cavity of a gill pouch. The appropriate attachment organelle in this case is one which will ensure tightness of grip.

It should be noted that these highly interesting ciliates have all been described from bottom-dwelling littoral animals, albeit from widely separated localities. Further studies may reveal their more extensive geographical distribution, and are in any case required before it can be determined whether the resemblances between *Ellobiophrya* and *Caliperia* are due to convergence operating upon the products of the two distinct scyphidian stems—as is suggested by the fact that the endoplasm of the zooid is continuous with that of the caudal processes in the former genus but not in the latter one—or to descent from a common stock otherwise obscured by specialized adaptations to very different hosts.

Acknowledgments

Thanks are due to Dr. J. L. Hart, Director of the Biological Station, Saint Andrews, N. B., and to his staff, not forgetting Mr. W. G. Carson, Captain of the M/B "Mallotus", for facilities extended during my survey.

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ISOLATION OF CORTISONE AND CORTISOL FROM THE PLASMA OF PACIFIC SALMON (*ONCORHYNCHUS NERKA*)

Sir:

Cortisol (I) has been identified as the principal adrenal-cortical hormone in the peripheral plasma of the normal human[1] where it occurs at a concentration of approximately 10 μg. per 100 ml.[2] It is also the principal steroid in the blood of several animals[3] and has been tentatively identified in carp plasma.[4] Attempts to find cortisone (II) in adrenal vein[5] and peripheral human blood[6] have been unsuccessful. Occasional blood samples have been reported to contain small amounts of a "cortisone-like" substance,[1,7] but the evidence is inadequate to establish identity.

We wish to report the isolation of I and II from Fraser River sockeye salmon (*O. nerka*) just prior to their arrival on the Adams River spawning grounds. Blood was obtained from (*ca*). 100 fish by severing the caudal artery. Ethyl acetate extractable steroids were obtained from 1760 ml. of plasma by the usual procedures. Chromatography on purified Whatman no. 1 paper was performed in three solvent systems known to resolve I and II from closely related steroids; ($CHCl_3$:$C_6H_6$1:1–formamide (III), toluene–propylene glycol (IV) and butyl acetate:ethylene glycol:H_2O 20:1:1. The corresponding U.S.P. reference standards and I and II were located on chromatograms both by alkali fluorescence and the reduction of blue tetrazoleum. When substance I was treated with sulfuric acid at 22° for 2 hr. it exhibited the same spectra as U.S.P. I with $\lambda_{max.}$ at 237, 282, 395 and 478 mμ and O.D. ratios of 0.98:1:0.47:0.55 and II gave the same spectra as cortisone with $\lambda_{max.}$ at 284, 343 and 420 and O.D. ratios of 1:0.38:0.31. The concentration of I and II was determined by the quantitative Porter–Silber[8] test for the dihydroxyacetone side-chain and each gave typical spectra with $\lambda_{max.}$ at 410 mμ. Substance I gave ϵ242 mμ 15.8 × 10^3 (MeOH) and II gave ϵ237 mμ 15.8 × 10^3.

The dihydroxyacetone side chain of I and II was oxidatively removed with $NaBiO_3$.[1] The ring oxidation products of I and II were inseparable from authentic 11β-hydroxy-Δ^4-androstene-3,17-dione and Δ^4-androstene-3,11,17-trione respectively in both benzene–hexane:methanol:water[1] and heptane:benzene 1:1–formamide, each of which gives excellent resolution from closely related 17-ketosteroids. The yield of both oxidation products was comparable to that obtained from U.S.P. steroids and both gave positive tests for the Δ^4-3 ketone and 17-keto groups.

Homogeneous II was obtained after a single chromatogram in solvent III, whereas I was resolved further in solvent IV. The recovery of cortisol (I) was 17 μg. and cortisone (II) 37 μg./100 ml. of plasma, with an over-all recovery of *ca.* 60–70%.

At full sexual maturity Pacific salmon show extensive degeneration of the pituitary and a marked hyperphasia of the adrenal gland, and this has been postulated to play a dominant role in the degenerative changes and death of the fish after spawning.[9] The high levels of I and II lend support to the suggested high secretory activity of the gland. The plasma levels of I and II at various stages of sexual maturity will be reported elsewhere.

Compounds I and II exhibited infrared spectra identical with cortisol and cortisone, respectively.

Acknowledgment.—We are grateful to Beckman Instruments, Inc., for recording the infrared spectra of the micro size samples.

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Seaweedy Odours in Cod and Haddock

by C. H. CASTELL

Fisheries Research Board of Canada
Technological Station, Halifax, N.S.

Introduction

ODOURS described as "seaweedy" are frequently encountered in the flesh of relatively fresh cod and haddock. They suggest the odour often observed from seaweed-covered rocks at low tide. They also are quite similar to the odour of iodine.

"Seaweedy" odours are usually associated with fish that are in a prime state of preservation. Dr. Shewan and his associates at the Torry Research Station in Scotland have developed a very comprehensive score sheet for the organoleptic grading of "White" fish. In this score sheet they have given 10 points out of 10, both for odour and flavour, when the fish are characterized as being "seaweedy".

Many of our experienced fishermen support this view: "The seaweedy odour is the normal odour of fresh fish". They supplement this by: "This seaweedy odour is more pronounced on fish from certain banks and at certain times of the year". Some of our inshore fishermen have found that fish from one particular bay or inlet are "seaweedy" while those caught in an adjacent area are not. They believe that the seaweeds among which the fish are feeding are responsible for the odour. Most of these fishermen are firmly convinced that when the odours do occur, they are present in the muscle of the fish at the time they are taken from the water and are not in any way associated with spoilage.

A somewhat different view is that "seaweedy" odours are the result of specific types of food that fish have been eating. For example, the Norwegians have found that codfish in some of the fjords of Greenland eat large quantities of one species of snail. These fish develop strong seaweedy and iodine-like odours which frequently become so unpleasant that they are quite unfit for human food.

It is, of course, impossible to tell whether the seaweedy odours observed by our local fishermen are identical with those described by the Norwegians for these Greenland cod. It suggests however, that feed, rather than the presence of seaweeds in the feeding ground, may be the responsible factor.

The fish plant foremen add to this confusion by saying that they occasionally have vessels bringing in a whole catch with fish almost unusable because of strong seaweedy odours.

These observations stir up in our mind a series of questions regarding the nature of seaweedy odours and the mechanism by which they are formed: Are they ever the natural odour of normal fish? Do these odours decrease or increase after the fish have been taken from the water? Will certain types of feed predispose our fish to deve'op these seaweedy odours? Do bacteria in any way contribute to their development? Are they affected by the storage temperature of the fish? Do they tend to become more prevalent or more intense during certain seasons? And, of course, the practical man wants to know whether they contribute seriously to deterioration in the quality of fresh or processed fish?

This article has been written for two reasons. First, to give the results of some of our own tests and observations related to fish with seaweedy odours; and second, in the hope that it will stimulate others, and particularly the fishermen and fish processors to give their views on this problem and their experiences with seaweedy fish.

During the past five or six years at the Technological Station of the Fisheries Research Board of Canada, Halifax, we have given a great deal of attention to the odours that deve'op during the early stages of spoilage in cod and haddock. At one time we took it for granted that seaweedy odour was a natural characteristic of these species and an indication of freshness. However, many observations, particularly with haddock, have changed our ideas regarding the significance of this odour. We now believe that seaweediness may not always be synonymous with freshness, but may actually be a symptom of one of the earlier stages of spoilage, and at times it may have very definite commercial significance both for fresh and frozen fish.

Development of seaweedy odour after the fish have been caught.

For purposes quite apart from the problem of seaweedy odours, we have graded and measured the quality of a large number of fillets cut from fish immediate'y after discharge from the trawlers. The records not only give the characteristic odours observed on each individual fillet, but they also show the number of days each fish was iced in the hold before landing. The results of 1020 of these examinations are given in Table 1. It may be seen that in this particular group of fish the seaweedy odours did not begin to develop until the fish had been stored in ice for more than 3 days. They occurred at approximately the same stage as fish that were described as having "not much odour". Not shown in the Table is the fact that these odours were described as "slightly seaweedy" or "trace of seaweedy" during the earlier storage periods and "seaweedy" and occasionally "strong seaweedy" as the storage period increased. Several fish after 7 and 8 days of storage were described as being "strongly seaweedy and slightly sour". The succession of odours as the storage period progressed appears to be:—fresh fish odour—not much odour — seaweedy — sour. None of these fish had reached later stages of spoilage where the putrid and other obnoxious odours begin to develop.

Trimethylamine (TMA) values and seaweedy odours

During the first 7 or 8 days that gutted cod and haddock are stored in ice there is a slow but continuous rise in their TMA content. There is none or only a trace at the time the fish are caught. This increases to values of 3 to 5 for well iced fish that are 7 to 9 days in storage.

It is interesting therefore, to compare the TMA values and odours that have been recorded for cod and haddock fillets. Examples of this are given in Tables II and III. Table II shows

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the frequency distribution at various TMA levels between 0 and 30 for 237 fillets cut from fresh and spoiling gutted fish immediately after they had been discharged from the trawler. These fillets had been recorded as either "fresh", "not much odour", "seaweedy", "seaweedy and sour", or "sour". Table III gives similar data from another lot of 751 fillets that were examined at another time.

It may be seen that in the first of these lots of fish (Table II) 75% of the fillets recorded as being "seaweedy" were in the TMA range between 0.5 and 3.0 and in the second lot (Table III) 80% of the seaweedy fillets were between 0.6 and 4.0. In contrast to this, 80% of the fillets recorded as "very fresh" in the first lot had TMA values under 0.5; in the second lot 82% of the "very fresh" fillets were under 0.6. It should be noted that in both lots of fish a small percentage with seaweedy odours had very low TMA values. In every case the records show that for these particular fillets th actual description of the odour was "slightly seaweedy".

Seaweedy odour and season

During June, July and August the incidence of seaweedy odours was greater than for the three months previous. During March and April between 20 and 30% of the fillets cut from the fish that had been iced in the boat for 4 days were recorded as being "seaweedy" or more often "slightly seaweedy". Comparative figures for the month of June show that 75% of the fillets from the four day fish were found to have these odours. The number of fillets with "strong" or "very strong seaweedy" odour was much greater during the summer months than in the late winter or early spring.

We have no records or observations on seaweedy odours from fish caught during October, November, December and January. So far, everything seems to indicate that these particular odours

TABLE I. The predominating odours of 1020 haddock fillets cut from gutted fish that had been stored in ice in the trawler from one to nine days at the time of landing. These fish were all from the same vessel but not all from the same trip.

FILLET ODOUR	Total No. of fish	Number of fish in each age group from 1 to 9 days.								
		1	2	3	4	5	6	7	8	9
Fresh	122	16	16	42	32	6	0	5	5	0
Not much odour	248	0	0	0	50	23	74	78	17	6
Seaweedy	353	0	0	0	65	88	95	84	21	0
Sour	224	0	0	0	4	0	35	36	62	87
Offensive odours	74	0	0	0	2	4	6	26	22	14

TABLE II. Percentage distribution of fillets recorded as being "fresh", "not much odour" (N.M.O.), "seaweedy" (S.W.), "seaweedy and sour" (S.W. + Sour) and "sour" at various TMA levels between 0 and 30. The fillets were examined immediately after discharge of the gutted fish from the vessels. They represent 237 fish.

TMA LEVEL	PERCENTAGE				
	FRESH	N.M.O.	S.W.	S.W. + SOUR	SOUR
0 — 0.25	24	0	8	0	0
0.26 — 0.5	56	15	17	0	8
0.51 — 1.0	20	34	23	8	21
1.1 — 2.0	0	37	47	25	21
2.1 — 3.0	0	6	5	17	6
3.1 — 4.0	0	6	0	0	9
4.1 — 5.0	0	0	0	17	21
5.1 —10.0	0	1	0	33	9
10.0 —15.0	0	0	0	0	2
15.1 —20.0	0	0	0	0	2
20.1 —30.0	0	0	0	0	0

TABLE III. Numbers of fillets and percentage distribution at various TMA levels of fillets recorded as "fresh", "not much odour" (N.M.O.), "seaweedy" (S.W.), "sour" and "very offensive odours" (V.O.). These fillets had been cut from 751 gutted fish as they came from the trawlers.

TMA RANGES	FRESH		N.M.O.		S.W.		SOUR		V.O.	
	No.	%	No.	%	No.	%	No.	%	No.	%
0 — 0.20	11	15.3	0	0	10	3.7	0	0	0	0
0.21 — 0.40	29	40.3	23	9.2	13	5.8	0	0	0	0
0.41 — 0.60	19	26.4	33	13.2	30	10.1	0	0	1	1.2
0.61 — 0.80	9	12.5	48	19.2	33	12.2	2	2.7	9	10.4
0.81 — 1.0	3	4.2	45	17.0	21	7.8	1	1.3	4	4.6
1.1 — 2.0	1	1.4	82	32.8	112	41.2	13	17.6	22	25.5
2.1 — 3.0	0	0	15	60.0	47	17.5	22	29.7	24	27.8
3.1 — 4.0	0	0	2	0.8	5	1.8	26	35.3	13	15.1
4.1 — 5.0	0	0	0	0	0	0	6	8	7	8.2
5.1 — 10.0	0	0	0	0	0	0	4	5.3	6	7.0

TABLE IV. The number of fillets having "slightly seaweedy" (Sl. S.W.) "seaweedy" (S.W.) and "strong seaweedy" (St. S.W.) odours during storage periods between 2 and 8 days inclusive for lots of stored fish. Ten fish were examined daily from each of the 3 lots of fish. All fish were held in ice, but one lot was also wrapped in paper and stored in water tight aluminum containers.

Days Storage	Iced Fillets			Wrapped Fillets			Gutted Fish		
	Sl.S.W.	S.W.	St.S.W.	Sl.S.W.	S.W.	St.S.W.	Sl.S.W.	S.W.	St.S.W.
2	0	0	0	3	0	0	6	0	0
3	0	0	0	7	1	0	4	2	0
4	0	0	0	0	0	0	0	7	1
5	3	0	0	0	0	0	8	1	0
6	1	0	0	0	2	0	0	2	3
7	0	0	0	0	0	0	0	0	3
8	0	0	0	0	0	0	0	0	0

SEAWEEDY ODOURS QUESTIONNAIRE

1. Have you ever encountered cod or haddock that had a distinct seaweedy odour?
   Answer:

2. Have you any reason to believe that this particular odour was present at the time the fish were caught?
   Answer:

3. Have you found that fish caught in any particular area had a greater tendency to become seaweedy than the ordinary run of fish?
   Answer:

4. Have you observed that seaweedy odours in fish are restricted to certain seasons of the year? If so, at what season?
   Answer:

5. Have you found that fish with seaweedy odours have any other characteristic that distinguishes them from non-seaweedy fish? Are they more slimy? Do they soften more rapidly?
   Answer:

6. Have you any reason to believe that the type of feed taken by the fish is related to the development of this odour?
   Answer:

7. Have you ever observed that seaweedy odours become stronger after the fish have been stored for a while?
   Answer:

8. Have you ever encountered a whole catch that unexplainably became seaweedy?
   Answer:

9. If you are a processor, have you encountered or been troubled with seaweedy odours in frozen fish?
   Answer:

10. Have you ever observed that frozen fish with seaweedy odours becomes very unpalatable when heated or cooked?
    Answer:

11. Have you had any other experiences with seaweedy odours in fish or fishery products that you consider might contribute to our understanding of this problem?
    Answer:

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are more prevalent and often much more intense during the warmer summer months.

Location of the seaweedy odour in the fish

Enquiries among those who handle the fish in the industry have indicated that "seaweedy" odours are almost entirely absent from the fish in the hold of the vessel. In contrast, they are quite familiar to those who handle the fillets in the plant.

Examination of the fillets shows that, when present, the seaweedy odour is associated with the outer side of the fillet rather than the side adjacent to the gut cavity. This raises the question whether the skin itself, or the slimes associated with the skin, causes or contributes to this particular odour. Is there a difference in the development of seaweedy odour in fish muscle from which the skin has been removed?

To answer this question partially, we obtained 200 well-iced trawler-caught cod that had been less than 2 days in the vessel. One hundred of these fish were buried in ice. The other 100 fish were filleted, giving 200 fillets. One hundred fillets were left unwrapped and buried in ice, while the remainder were placed in water-proof containers and these in turn were buried in ice. Each day ten fillets from each of these three lots were removed from the ice and carefully examined. The gutted fish were filleted before being examined. As the storage period increased the usual variety of spoilage odours developed, but for the sake of clarity only the seaweedy odours have been recorded in Table IV. The results show that more fillets have seaweedy odours and that the intensity of the odour is greater in fillets cut from the fish stored as gutted fish. They also indicate that the odour increases in strength with storage up to 6 or 7 days, at which time the first sour odours begin to develop.

Seaweedy odours and temperature

Fish caught during June and July were purposely held at temperatures ranging from 50° to 75°F. before being iced. The result was a marked increase in the intensity of the seaweedy odours compared with the controls that were iced at once. In some instances the fish subjected to these higher temperatures, developed odours which were even more disagreeable than the ordinary seaweedy types. Once again these odours were most evident in the tissue immediately under the skin.

The foregoing remarks applied to off-shore fish. However, during these past few months we have also run

across instances of strong seaweedy odours developing in relatively fresh, in-shore cod and haddock that had not been iced. These fish subsequently became soft and the odours were so objectionable that the fish had to be discarded.

Taken altogether, we believe that these facts and observations suggest the following:—

1. That seaweedy odours are not an indication of really fresh fish, but if they develop they generally do so in iced fish prior to the formation of the first odours known to be caused by bacteria.

2. Shortly after the fish become slightly sour the seaweedy odour disappears.

3. In most trawler-caught fish the seaweedy odours begin to develop on the 4th or 5th day and disappear as bacterial activity produces the first definite spoilage odours between the 6th and 8th day.

4. More fish caught during the summer months develop seaweedy odours than those caught during the spring or fall; and the odours are stronger in the summer-caught fish. There is some evidence that they may develop at an earlier period in fish caught during the summer.

5. High holding temperatures, immediately after the fish have been caught, accentuate the development of these odours.

6. It would seem probable that uniced, shore fish, especially those caught during the summer months would be more subject to this complaint than iced trawler-caught fish.

7. It also seems probable that under certain circumstances, environmental conditions may permit the reactions responsible for the seaweedy odours to develop to an extent where the fish become extremely objectionable.

8. If this excessive development of the seaweedy condition occurs the objectionable odours are more readily observed when the temperature of the fish is raised considerably or when the fish is cooked.

9. The seaweedy odours are not usually observed by examining the whole or gutted fish. They are most noticeable in the tissue immediately under the skin.

10. At the present time we have not the slightest idea of the nature of the compound giving rise to these odours nor of the mechanism by which they are produced. Some of the facts suggest that the odour may be the result of the action of a tissue enzyme, and perhaps that oxidation of lipid material immedately under the skin is involved.

11. There is every evidence that lowering of the holding temperature immediately after the fish are caught will retard the development of the seaweedy odours and prevent the more serious conditions that sometimes develop.

This article is a progress report and not the result of a completed investigation. Much remains to be learned about seaweedy odours in fish. Our investigations will continue during the coming year. Among the readers of this article there may be persons who have had experience with this odour, either as biologists, fish inspectors, fishermen, fish processors or consumers. We would appreciate receiving whatever additional information regarding any phase of this problem these readers might have. In particular we would like to obtain further information on the incidence of seaweedy odours in fish from in-shore fisheries and whether it appears to be a seasonal phenomenon.

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FROZEN FISH STORAGE

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Low temperature storage of frozen fish prevents the bacterial spoilage associated with deterioration of iced fish but other changes occur in the frozen product which may render it unpalatable to the consumer unless optimum freezing and storage conditions are used. Here only the actual requirements for good storage life will be considered assuming that we are provided with a reasonably good frozen product.

Most fish are more sensitive than meat to changes in storage. Fatty fish, such as herring, mackerel and salmon, differ markedly from the non-fatty species, such as cod and haddock. The fatty fish do not store well; fish fat is very susceptible to oxidation and rancidity develops early in the storage period. Exclusion of air by glazing or by wrappings impermeable to air, and the use of anti-oxidants, coupled with very low storage temperatures, near −30°F, may allow storage periods up to 6 months and sometimes longer.

The lean whitefish, of which group cod and haddock have been mainly studied, behave differently. Fat spoilage is important only in the later stages of the storage life. Prior to this, a less visible change occurs which is commonly termed development of dryness or toughening. It is due to denaturation of the protein in the fish flesh. One of the characteristics of this protein is its ability to bind the water in the flesh, and when it is changed or denatured this ability is lost. On cooking, the fish then tastes as if it were dried out; it is fibrous and chewy. One of the simplest ways of measuring these changes is by cooking the stored fish and assessing its quality by tasting and eating it.

The storage life of frozen packaged fillets stored at various temperatures determined by such a procedure is shown in the Table.

It must be emphasized that these figures are for fish of good initial quality. The use of fish of poor initial quality may shorten the storage period appreciably.

The influence of temperature on the storage life of frozen cod is very marked. Temperatures of −10°F. or lower are obviously necessary for reasonable cold storage life. For instance, some British workers suggest that at −20°F., cod no more than 24 hours on ice prior to freezing, remains nearly as good as fresh up to 8 months and may keep as long as 4 years before becoming inedible.

The times given in the above Table are those for which quality of the samples was judged to have become unacceptable and approaching inedibility. Thus in considering these maximum keeping times it should be emphasized that before the maximum is reached the quality has already deteriorated markedly from the fresh frozen quality. If frozen fish is to become a good quality product from the consumer's viewpoint, then the quality should not be allowed to deteriorate to these levels. The industry needs a product superior to the barely edible level to survive in today's frozen food field.

In any discussion of storage requirements it must be remembered that adequate packaging or protection to prevent drying out must be provided. Here, of course, the problem is much simpler if jacketed storages are used in which case the relative humidity can be kept at a much higher level.

Temperature Fluctuations During Handling and Transportation

The storage temperature in the larger cold storage units at the Atlantic Coast is usually about −10°F. However, the product is then transported long distances to

STORAGE LIFE OF FROZEN FISH

Species	Temperature of Storage			
	15°F.	10°F.	0°F.	−10°F.
	Months	Months	Months	Months
Cod	1.5 – 2	3.5 – 4	6 – 8	9 – 10
Haddock	—	3	—	10
Flatfish		[illegible]		
Rosefish	—	7	—	12
Halibut (Atlantic)	—	8	18	18

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the market where it may also be held for considerable periods before distribution to the retailers and ultimate consumers.

Until recently, no information was available on the effect of temporary storage at higher temperatures. This could happen during transit, either in railway refrigerator cars or in refrigerated trucks. Also temperature rises occur during loading and unloading.

It has now been found that if a period of storage at 0°F. is interrupted by a short period at a higher temperature, an immediate deterioration in quality of frozen cod fillets occurs and the storage life is shortened. Such an interruption by 2 weeks at 15°F. resulted in shortening of the normal storage life of 6-8 months by 2 to 2½ months. A shorter period of interruption, 3 days at 15°F., still resulted in a loss of storage life of about 1½ months.

This raised the question of what temperature must be maintained in the refrigerator cars and trucks during transportation to avoid serious deterioration and loss of storage life.

When storage of frozen cod fillets at 0°F. was interrupted by 2 weeks at 10°F., a reduction in storage life of about 2½ months occurred. Even with the interruption by storage at 5°F. there was a decrease of about a month.

Other experiments were carried out with cod fillets stored at −10°F. After a month at this temperature, they were divided into several lots. One was placed at 10°F., another at 5°F. and a third at 0°F., for a two week period. Subsequent storage was again at −10°F. The control samples, kept continuously at −10°F., had a storage life of 9 months. The samples interrupted by storage at +10°F. had a reduced storage life of only 4½ months. Those samples raised to +5°F. and to 0°F. showed much less deterioration, reaching the rejection level at about 6 to 7 months and about 8 months respectively.

It is evident that if fish is to be stored at −10°F. or 0°F. that transportation temperatures of +10°F. or +15°F. will result in a significant shortening in the storage life. Shipment at these temperatures results in approximately halving the storage life. It must be realized that long before the rejection point is reached there has been a reduction in quality. This occurs much earlier in the case of the fish exposed to transportation at temperatures higher than 0°F. to +5°F.

Results with a shipment temperature of +5°F., show much less reduction in keeping time. Similar results were obtained for a shipment temperature of 0°F. with fillets stored at −10°F.

It is recommended that the maximum temperature in refrigerated carriers be 0 to 5°F. It should also be noted that the product should be loaded and unloaded under conditions which will not raise its temperature above this level.

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Automatic Multistage Semimicro Zone Melting Apparatus

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THE technique of zone melting, the mathematics (*1*, *9*) and principles (*2*) of which have been adequately described, is not entirely new in metallurgical purification (*3*, *9*, *15*), but is relatively new in organic chemistry. Röck (*11*) has purified benzene; Hesse and Schildknecht (*6*), fatty alcohols; and Handley and Herington (*5*), benzoic acid, pyrene, anthracene, and crysene. The limited success of chromatographic techniques in the sterol field suggested that a suitable apparatus might make zone melting applicable to problems in this laboratory.

This method for purifying organic compounds requires that a narrow molten band travel slowly down a vertical column of the solid substance in a glass tube; impurities are separated and normally concentrated at the bottom of the column. A horisontal tube may be used in metallurgy (*5*), but in organic separations when the molten zone passes and the material crystallizes the solid drains away from the side of the tube and seeps back.

No available apparatus (*3–7*, *11*, *12*) was entirely suitable for the purpose. An automatic macromodel (*5*) was investigated, but the disengagement of the drive motor by the system of pawl and ratchet was not clearly understood, and it did not appear suitable for adaptation to a micro scale. A zone melting apparatus could have been modified by changing the heater carriage to a sample carriage and building a bank of heaters through which the sample tubes could pass, but the apparatus described here was completed before the advertisement appeared (*10*). An apparatus (*7*) apparently similar to the commercial one has been described, but the mechanical operations are different. The apparatus described here is cheaper, smaller, and self-contained.

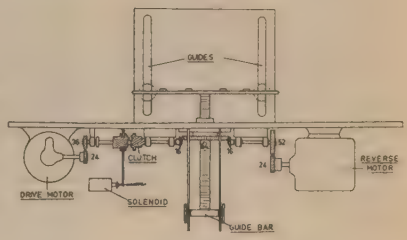
PRINCIPLE OF OPERATION

The apparatus (Figure 1) consists of a drive motor which raises a platform that supports four tubes containing the material to be purified. As the platform rises, the tubes pass through a heater which creates a well defined molten zone of material. The molten zone passes slowly down the column to the bottom. The platform reverses, travels quickly to its starting position, and again commences its slow ascent.

The slow drive speed for raising the platform is attained by a synchronous motor with a drive shaft speed of 120 revolutions per hour. This is reduced by external gears to 20 revolutions per hour. The last reduction is by a bevel gear with an internally threaded hub (Figure 2), through which passes a $^3/_8$-inch-diameter rod with 16 threads to the inch, fixed by a guide bar attached at right angles to the rod and sliding in a slot so that it cannot rotate. The bevel gear on completing 16 revolutions raises the platform 1 inch. The gear turns 20 times in 1 hour; thus the platform travels 1.25 inches per hour.

Figure 1. General layout of mechanical components

Drive motor, Bodine Type NSY-12R
Reverse motor, Bodine Type CL-2
Clutch solenoid, GE magnetic switch solenoid 16252
Bevel gears, two-16-tooth, one-64-tooth
Spur gears, two-24-tooth, one-36-tooth, one-52-tooth



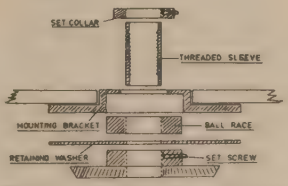


Figure 2. Exploded view of bevel gear assembly

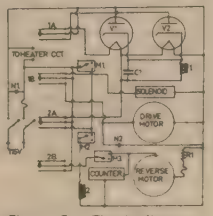


Figure 3. Circuit diagram of apparatus

Figure 4. Heater circuit

AUTOMATIC RECYCLE. On reaching a predetermined height the guide bar attached to the bottom of the threaded rod lifts a lever which operates a microswitch. The power is cut off from the drive motor, which is then disengaged from the system through the action of a solenoid-operated dog clutch. The reverse motor cuts in, returning the platform, in 6 seconds, to its original position, where on striking another microswitch it is stopped. After about 15 seconds the clutch engages and the drive motor starts. The delay before commencement of the recycle is effected by incorporation of a delay mechanism in the circuit, installed to avoid damage to the gear system.

OPERATION OF APPARATUS

When the electronic delay circuit (Figure 3) is switched on, 15 seconds elapse before the relay coil, 1, is energized, cutting off, through the action of relay contact 1*A*, the filament voltage to tube *V*1 and making the plate voltage to tube *V*2. The relay is held on until the circuit to the coil is broken, at which point even immediate application of current to the circuit would not energize the coil until 15 seconds had elapsed —the time required for the filament of tube *V*1 to heat up and allow sufficient current to flow to energize the coil.

Relay contact 1*B* controls the action of the clutch solenoid and the drive motor. When coil 1 is not energized, the clutch solenoid is in operation. This disengages the drive motor from the system and closes microswitch *M*3, which is so positioned that the circuit to the reverse motor is not complete unless the clutch is fully disengaged. Even with the clutch disengaged the reverse motor will not act until its circuit has been completed. This can occur only in the following manner. Microswitch *M*1 is closed either by action of the bar attached to the threaded rod or by manual manipulation. Coil 2 is then energized and relay

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contacts 2A and 2B close and break the circuit to the delay unit through relay contact 2A. When the circuit to the delay unit is broken, 2A makes a circuit to its own coil; this is necessary, as microswitch M1 is opened as soon as the reverse motor comes into operation. Relay contact 2B makes a circuit to microswitch M3. When the filament of tube V2 has cooled sufficiently to cut off the power supply to coil 1, relay contact 1 opens, the drive motor stops, and the clutch disengages and completes the circuit through M3 to the reverse motor. The rod then travels down until the bar strikes microswitch M2, opening the self-holding circuit to coil 2. 2A and 2B open, the reverse motor stops, the circuit to the delay unit is made, and after 15 seconds coil 1 is again energized. The clutch engages, the drive motor starts, and a new cycle commences.

CONSTRUCTION OF APPARATUS

The mechanical components were assembled on an aluminum plate 10 × 24 × 3/8 inches. The bevel gear assembly was first manufactured and assembled and then mounted in the center of the plate. The reverse motor gear shaft was assembled, and the 16-tooth bevel gear was fastened to one end of a 2-inch length of 1/4-inch stainless steel shaft, by means of a pin driven through the hub of the gear and through the shaft. The 52-tooth spur gear was fastened to the other end by drilling a 1/4-inch hole in the gear and fitting it to the shaft, and a small hole was drilled parallel to the shaft so that it cut a keyway from the shaft and the gear. The hole was then tapped and a screw inserted which held the shaft and the gear firmly together. The gear assembly was mounted on 1/4-inch brass pillow blocks, fastened to an aluminum mounting block of such thickness that the bevel gears meshed correctly. The slow-speed drive motor shaft was mounted by a similar procedure. A hub was brazed to each spur gear which was to be mounted on a motor shaft, a hole drilled and tapped on each hub, and a setscrew inserted. The gear was held in position on the drive shaft of the motor by the setscrew.

This allows easy mounting, simple adjustment, quick removal, and disengagement of any motor while tests are being carried out on the correct positioning of the electrical components of the system. If the gear system is not mounted properly and any tightness occurs, difficulty will be experienced in disengaging the clutch; careful alignment is necessary and the clutch teeth should be angled as much as possible. Microswitches M1 and M2 can be mounted in many ways. One convenient mount is on the side of a right-angled bracket, mounted on the outside of the guide bar support. A slot is cut in the side of the bracket to allow easy positioning of the microswitches. The mechanism of the microswitch is actuated through coming in contact with that part of the guide bar which protrudes through the guide bar support.

Each heater unit is constructed from a single loop of No. 26 bare nickel-chromium wire attached to copper rods approximately 5 inches long and 1/8 inch in diameter, and held in place by a Bakelite strip which provides both insulation and a means of adjusting the height of the heater. Each heater is controlled by a rheostat, wired in the manner of a voltage divider (Figure 4). The type of circuit used gave the best individual control for each heater. With all the heaters working at maximum temperature the load is about 13 amperes. However, the 6-volt center-tapped 10-ampere Hammond transformer worked satisfactorily and there was no indication of heating due to overloading.

To determine the effectiveness of the apparatus a trial run was carried out with mixtures of 1 and 0.1% 2,4-dinitrophenylhydrazine in naphthalene. Approximately 500 mg. of the mixture was heated and drawn into a preheated tube 25 cm. long (outside diameter 4 mm., inside diameter 2 mm.), to a height of about 15 cm. Five passes completely removed the 2,4-dinitrophenylhydrazine from approximately 90% of the 0.1% mixture and concentrated it in a 1-cm. zone above the point which the heater reached at the end of each run. Fifteen passes were required to remove the impurity completely from approximately 80% of the 1% mixture, concentration taking place in the bottom 3 cm. of the tube.

Table I. Values of Component Parts of Electrical Circuit

	Resistors
R1	100-ohm 50-watt
R2, R3, R4, R5	2-ohm 50-watt rheostats
R6, R7, R8, R9	Loop of No. 26 bare nickel-chromium wire
	Condensers
C1	50-mfd. electrolytic
V1, V2	117Z6GT, tubes converted to ordinary triode, doubling current-carrying capacity
	Relays
1	P & B Relay, KA 11D, 110-volt, d.c., D.P.D.T.
2	Guardian Relay, Series 200, 115-volt, a.c., D.P.D.T.
	Switches
M1, M2, M3	Unimax. Type 2 HBW-1, microswitch
On-off	10-ampere D.P.S.T. toggle switch
	Transformers
T1	Hammond, Type 1129R-60CY, 6-volt, 10-ampere center tapped
	Fuses
	Two 2-ampere, Type 3-AG
	Indicator Lights
N1, N2	Neon bulb, Type GE-NE 51, resistor-type mountings

ACKNOWLEDGMENT

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LINGCOD MUSCLE NUCLEASE[1]

NEIL TOMLINSON

Abstract

A nuclease from muscle of lingcod (*Ophiodon elongatus*) has been purified by chromatography on diethylaminoethyl cellulose in the free-base form by stepwise elution with increasing concentrations of tris(hydroxymethyl)aminomethane. HCl, pH 7.0. The nuclease has been shown to hydrolyze ribonucleic acid, adenosine 3′-benzyl phosphate, thymidine 3′-*p*-nitrophenyl phosphate, and thymidine 5′-*p*-nitrophenyl phosphate. The latter compound was hydrolyzed at a very low rate. It did not hydrolyze adenosine 2′- or 5′- benzyl phosphate or certain nucleoside cyclic phosphates. The enzyme was inhibited by monoiodoacetate but not by heparin.

Introduction

The partial purification and some of the properties of an enzyme from the muscle of lingcod (*Ophiodon elongatus*) have been described (1). This enzyme degraded ribonucleic acid (RNA)* with the release of nucleoside 3′-phosphates. The present work describes the further purification of the enzyme by chromatography on DEAE, and the results of an investigation of the enzyme's substrate specificity.

As it has been found that the purified enzyme can hydrolyze certain simple esters of both ribo- and desoxyribomononucleotides, it will in future be called a nuclease, rather than a ribonuclease as in the previous publication (1).

Materials and Methods

The procedure for the preparation of muscle extracts of lingcod suitable for chromatographic treatment was as previously described (1), except that partial purification by isoelectric precipitation at pH 4.5 was omitted and the extract was heated 10 minutes at 58° C at pH 7.0 rather than at pH 9.0.

Protein and all light absorption measurements were made as previously described (1).

Temperature of incubation in all enzymatic assays was 25° C.

Nuclease and phosphodiesterase assays of fractions from chromatographic columns were performed by methods similar to those used previously (1), but modified to provide greater sensitivity. Thus, for assay of nuclease, the reaction mixture contained sodium succinate buffer pH 6.4, 0.025 *M*, RNA (95 μg RNA-P), and 0.5 ml of the fraction assayed in a total volume of 1 ml. Aliquots

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*ABBREVIATIONS: DEAE, diethylaminoethyl cellulose; DNA, desoxyribonucleic acid; RNA, ribonucleic acid; RNA-P, ribonucleic acid phosphorus; T3′-NPP, thymidine 3′-*p*-nitrophenyl phosphate; T5′-NPP, thymidine 5′-*p*-nitrophenyl phosphate; Tris, tris hydroxymethyl aminomethane; TCA, trichloroacetic acid. 2′,3′-AMP, adenosine 2′,3′-cyclic phosphate; 2′,3′-UMP, uridine 2′,3′-cyclic phosphate.

(0.4 ml) of the reaction mixture were removed immediately after the addition of the enzyme and after a 2-hour incubation period. Each aliquot was at once mixed with 0.5 ml of MacFadyen's UTCA reagent (2) and 0.1 ml of water in a 75 mm × 10 mm test tube. The mixture was kept at room temperature for 30 minutes and was then centrifuged at 200 × *g* for 10 minutes. A 0.4-ml aliquot of the supernatant solution was carefully removed with a pipette and added to 0.8 ml of distilled water. The optical density of this solution at 260 mμ was determined with a suitable dilution of the UTCA reagent as reference blank. Reaction blanks with enzyme omitted were used. Results are reported simply as net increases in optical density at 260 mμ. Units of activity were not used in this work due to the impracticability of making corrections to compensate for the influence that different buffer concentrations of the various fractions assayed had on the enzyme's activity. Phosphodiesterase was assayed with bis (*p*-nitrophenyl)phosphate as substrate. The assay mixture contained sodium succinate buffer pH 6.4, 0.025 *M*, substrate 0.001 *M*, and 0.2 ml of the fraction assayed, in a total volume of 0.33 ml. After 4 hours' incubation, 0.8 ml of 0.1 *N* sodium hydroxide was added, and the *p*-nitrophenol released was determined as previously described (1).

Assays for enzymatic activity with *p*-nitrophenyl phosphate, T3′-NPP, or T5′-NPP as substrate were carried out as for phosphodiesterase activity. The time of incubation was reduced to 2 hours with certain exceptionally active fractions.

Assays for desoxyribonuclease activity were carried out by the method of Maver and Greco (3). The reaction mixture contained sodium succinate buffer pH 6.4, 0.025 *M*, DNA 0.5 mg/ml, and enzyme in a total volume of 0.4 ml. The incubation period was 4 hours.

Examination of the products of hydrolysis of RNA by the purified nuclease was made by means of two-dimensional paper chromatography and paper electrophoresis as before (1), but the nucleotides were eluted in 0.05 *M* sodium phosphate pH 7.0 rather than in 0.01 *N* HCl. The same procedure was used for quantitative measurement of nucleotides separated by chromatography or electrophoresis when the benzyl esters of the adenylic acid isomers or the cyclic ribomononucleotides (*vide infra*) were the substrates. For the latter the absorption data of Brown, Magrath, and Todd (4) were used.

The ability of the nuclease to hydrolyze 2′,3′-AMP and 2′,3′-UMP, and the influence of these two compounds on the hydrolysis of RNA by the enzyme, were investigated with a reaction mixture that contained either of the cyclic mononucleotides alone (0.006 *M*), or RNA (206 μg P/ml) with or without a cyclic mononucleotide (0.006 *M*), together with sodium succinate buffer pH 6.4, 0.025 *M*, chlortetracycline 5 p.p.m., and enzyme. Chlortetracycline does not inhibit either the nuclease or the phosphodiesterase at this concentration and was used to prevent bacterial growth in extended incubations. The total volume of the digestion mixture was 80 μl, and incubation was carried out in small, tightly stoppered test tubes. Controls without enzyme were used. Aliquots of 25 μl were removed at the beginning of the experiment and after 10 and 20 hours of incubation. When the mononucleotides alone were the substrate,

the aliquots removed were subjected to electrophoresis in sodium phosphate buffer pH 7.4, 0.2 *M* according to the method of Davis and Allen (5). When RNA with or without the mononucleotide was substrate, the aliquots were subjected to two-dimensional paper chromatography using isopropanol–water (70/30, v/v) with 0.35 ml ammonia solution (0.88 sp. gr.) for each liter of gas space in the tank (6) as the first solvent system, and with isobutyric acid 0.5 *N* and ammonium hydroxide pH 3.7 (7) as the second.

The ability of the enzyme to attack the benzyl esters of adenosine 2′-, 3′-, and 5′- phosphates was investigated in a manner identical to that used with the cyclic mononucleotides, including examination of the results by paper electrophoresis.

Column chromatography of proteins was carried out with jacketed columns refrigerated at 1° C and with the tubes in the fraction collector held in a bath of refrigerant at the same temperature.

DEAE type 20, capacity 0.85 meq/g was obtained from the Brown Co., Berlin, N. H. DNA was obtained from Worthington Biochemical Corporation, Freehold, N. J. Heparin (110 international units/mg) was obtained from the Connaught Medical Research Laboratories, Toronto, Ont. 2′,3′-AMP and 2′,3′-UMP, as the barium salts, were obtained from Schwarz Laboratories, Inc., Mount Vernon, N. Y. These two compounds were converted to their sodium salts by treatment with Dowex 50 (×8) in the sodium form before use. Adenosine 2′-, 3′-, and 5′-benzyl phosphates, T3′-NPP and T5′-NPP, and bis(*p*-nitrophenyl)phosphate were gifts from Drs. H. G. Khorana, J. G. Moffatt, W. E. Razzell, G. M. Tener, and A. Turner of the British Columbia Research Council, Vancouver, B.C. Disodium *p*-nitrophenyl phosphate was obtained from Sigma Chemical Co., St. Louis, Mo.

Results

The results of the chromatography of lingcod muscle extracts prepared as described above are shown in Figs. 1 and 2.

The DEAE used in the chromatogram of Fig. 1 was equilibrated with Tris.HCl, pH 6.9, 0.02 *M*, before the protein was added. Gradient elution was used to develop the chromatogram, the gradient being obtained by means of a single reservoir and 125-ml mixing flask. The mixing flask was filled initially with Tris.HCl, pH 6.9, 0.02 *M*, and the reservoir contained Tris.HCl, pH 5.8, 0.5 *M*. With this procedure, although three or four fractions containing nuclease were obtained free from phosphodiesterase activity (at least within the limits of sensitivity of the phosphodiesterase assay), separation of the nuclease from the phosphodiesterases was not good. The use of Tris.HCl, pH 5.8 or pH 7.0, 0.25 *M*, in the reservoir did not improve separation. In addition, it was observed that the buffered DEAE had a surprisingly low capacity for the protein contained in these preparations, for when 95 mg of protein, rather than 61 mg as in Fig. 1, was applied to a column of the same dimensions a much larger proportion of the total protein and a large part of the nuclease was not retained by the DEAE, indicating overloading. The

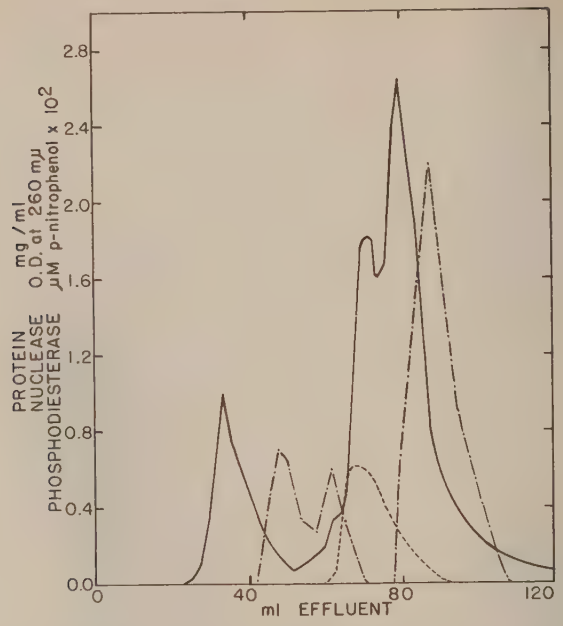

FIG. 1. Effluent diagram of protein preparation from lingcod muscle. Sixty-one milligrams of protein applied on 12 g DEAE previously equilibrated with Tris.HCl, 0.02 *M*, pH 6.9. Column 29 cm long × 18 mm diameter. Gradient elution with Tris.HCl (see text). Flow rate, 0.25 ml/min; fractions, 2 ml. First fraction collected after addition of protein. Hold-up volume *ca.* 38 ml. Protein ——. Nuclease----. Phosphodiesterase –·–·–.

capacity of DEAE in the free-base form for the protein was examined and was found to be very much greater than that of the buffered form. Mitz and Yanari (8) have reported the successful use of DEAE in the free-base form, with carbon dioxide solutions as eluent, in the chromatography of a number of proteins. In view of these facts, and the known moderate stability of the nuclease and phosphodiesterases under alkaline conditions (1), chromatography using DEAE in the free-base form and stepwise elution with increasing concentrations of Tris.HCl, pH 7.0, was investigated. Figure 2 presents the results. It is obvious that much better separation of the nuclease from the phosphodiesterases and greater purification of the nuclease with respect to protein were obtained. The three chromatograms show the influence of different degrees of loading of the DEAE with protein. In Fig. 2A the DEAE was very lightly loaded, in Fig. 2B it was slightly overloaded, and in Fig. 2C it was quite heavily overloaded. Moderate overloading can be used to advantage with the nuclease in order to obtain greater concentration of this activity in the effluent.

The nuclease activity accompanying the phosphodiesterase activity eluted with *M* Tris.HCl, and the small peaks of nuclease activity preceding the main nuclease peak in the overloaded column are regarded as artifactitious. Sober and Peterson (9) have pointed out the possibility of a condition such as this arising when stepwise elution is used. It is certain the nuclease and phospho-

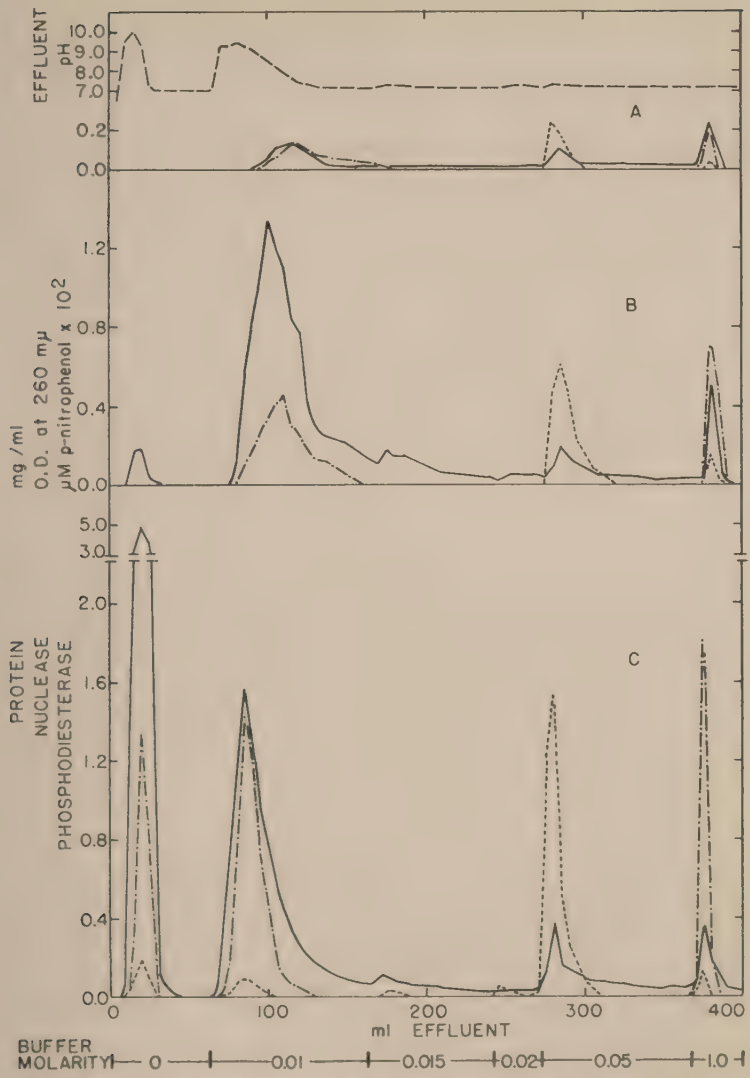

FIG. 2. Effluent diagrams of protein preparation from lingcod muscle. Each column contained 0.7 g DEAE in the free-base form. Protein applied: A, 14 mg; B, 75 mg; C, 120 mg. Column 2.5 cm long × 18 mm diameter. Stepwise elution, following distilled water wash, with Tris.HCl pH 7.0 at concentration indicated. Flow rate, 1 ml/min. First fraction collected during addition of protein. Hold-up volume *ca.* 6 ml. Fractions, 5 ml. Effluent pH for A – – –. Protein ——. Nuclease ----. Phosphodiesterase –· ·–.

diesterase eluted together are not identical, for a portion of each was obtained free from the other by chromatography on buffered DEAE (Fig. 1), and a preliminary experiment has indicated that the two can be separated on the free-base form by suitable intermediate steps in Tris.HCl concentration. It will be noted also that the quantity of nuclease activity eluted with the phospho-

diesterase by *M* Tris.HCl does not increase with increased protein added to the column as does the phosphodiesterase, presumably due to the displacement of the nuclease by more firmly bound material.

The effluent pH for the chromatogram of Fig. 2A is shown. For the chromatograms of Figs. 2B and 2C the changes in effluent pH are not shown, but were similar to those of Fig. 2A, reaching slightly higher values during addition of the protein and slightly lower values during the first passage of Tris.HCl through the column, with the peak value being displaced slightly to the left. The alkaline fractions were neutralized with cold *N* HCl immediately after they were collected.

The ability of the nuclease, following purification by either chromatographic procedure, to hydrolyze RNA, DNA, several simple esters of ribo- and desoxyribomononucleotides, bis(*p*-nitrophenyl)phosphate, and *p*-nitrophenyl phosphate has been examined.

The nuclease hydrolyzed RNA with the release of nucleoside 3′-phosphates, as previously reported (1), and hydrolyzed adenosine 3′-benzyl phosphate with the release of adenosine 3′-phosphate. However, using the assay conditions described above, with sufficient enzyme to provide over 90% hydrolysis of adenosine 3′-benzyl phosphate and hydrolysis of RNA to the extent that 70% of the RNA-P could be recovered as the nucleoside 3′-phosphates in 20 hours incubation, no hydrolysis whatever of adenosine 2′- or 5′-benzyl phosphates, of 2′,3′-AMP, or of 2′,3′-UMP could be detected. When either of the cyclic nucleotides was used together with RNA as substrate, 60 to 70% inhibition of the release of nucleoside 3′-phosphates was observed, with no detectable change occurring in the amount of the cyclic nucleotide. With adenosine 3′-benzyl phosphate as substrate no intermediate formation of the cyclic nucleotide could be detected, although this would have been readily found by means of the electrophoretic technique used if any appreciable amount had accumulated (5).

No hydrolysis of DNA, as measured by release of acid-soluble, ultraviolet-absorbing material (3), could be demonstrated, but the nuclease hydrolyzed T3′-NPP and T5′-NPP with the release of *p*-nitrophenol. The 5′-isomer was hydrolyzed at less than 2% of the rate of the 3′-isomer; 0.0005 μmole *p*-nitrophenol was released from the 5′-isomer against 0.031 μmole from the 3′- in 2 hours by 0.2 ml of a fraction from the main nuclease peak of Fig. 2C containing 138 μg protein/ml. It thus appears that the nuclease can hydrolyze phosphodiester linkages involving either ribose or desoxyribose. The very slow hydrolysis of T5′-NPP seems to be an exception to the apparent specificity of the enzyme for phosphodiester linkages in which the linkage not hydrolyzed must be to the 3′- position of ribose or desoxyribose. Possibly the *p*-nitro group influences the reaction in such a fashion as to modify the apparent specificity of the enzyme. A similar suggestion has been made by Schmidt (10) in connection with the activity of certain phosphomonoesterases. The possibility that the hydrolysis of T5′-NPP was due to a contaminating enzyme was considered. It cannot be due to contamination by the phosphodiesterases from which the nuclease appeared to be separated on the chromatogram. The

phosphodiesterase(s) preceding it from the column did not hydrolyze T5′-NPP, while the one following it did hydrolyze this compound, but was found not to be inhibited by 0.005 *M* monoiodoacetate, whereas the hydrolysis by the nuclease was inhibited almost completely. The fact that the nuclease purified by either chromatographic procedure hydrolyzed the same compounds, and that 0.005 *M* monoiodoacetate was found to inhibit the hydrolysis of each by more than 90%, suggested that one enzyme was probably concerned. No hydrolysis of either *p*-nitrophenyl phosphate or bis(*p*-nitrophenyl)phosphate could be detected. If the enzyme can hydrolyze either of these compounds it must be at a rate considerably less than 0.5% of that at which it hydrolyzed T3′-NPP.

The influence of heparin on the activity of the nuclease was investigated. Heparin is a powerful inhibitor of pancreatic ribonuclease (11, 12). At a concentration of 155 μg/ml, under assay conditions otherwise as described above, it produced no detectable inhibition of the lingcod nuclease with any of the substrates that the enzyme hydrolyzes.

Discussion

As has previously been pointed out (1) the lingcod muscle nuclease resembles Hilmoe and Heppel's (13) spleen nuclease fraction III (also called spleen phosphodiesterase (14)) in that they both hydrolyze RNA with the release of nucleoside 3′-phosphates, have similar pH optima and heat sensitivity, and do not require metallic ions for activation.

The present findings indicate several additional points of similarity. The spleen enzyme, like the lingcod nuclease, hydrolyzes ribomononucleoside 3′-alkyl phosphates but not the 2′- or 5′-esters (15), hydrolyzes T3′-NPP (16), and does not hydrolyze nucleoside 2′,3′-cyclic phosphates. Spleen nuclease fraction III hydrolyzed the nucleoside cyclic phosphates (15) but Hilmoe and Heppel subsequently freed it from this activity by means of an acetone fractionation procedure (16). However, in its apparent ability to hydrolyze T5′-NPP very slowly the lingcod nuclease differs from the spleen enzyme, with which no such activity has been detected. If the spleen enzyme does possess this activity, the rate of hydrolysis of T5′-NPP must be 0.01% or less of the rate for T3′-NPP (16).

The influence of monoiodoacetate and heparin on the activity of the lingcod enzyme is quite different from the influence these compounds have on the activity of the pancreatic ribonuclease. Monoiodoacetate is a very strong inhibitor of the lingcod enzyme with all the substrates yet known that it hydrolyzes, but the compound only inhibits the pancreatic enzyme in its attack on ribonucleic acid (17) and is without effect on its hydrolysis of simple esters of ribomononucleotides (5). Heparin inhibits all the activities of the pancreatic enzyme (11), but is without effect on the lingcod enzyme. It is interesting to speculate regarding the effect of heparin. It behaves as a competitive inhibitor of pancreatic ribonuclease (12, 18), and consequently its failure to inhibit the

lingcod nuclease suggests that the two enzymes have quite different active sites.

Acknowledgments

My sincere thanks are due to Drs. H. G. Khorana, J. G. Moffatt, W. E. Razzell, G. M. Tener, and A. Turner, British Columbia Research Council, for their very generous gifts of various nucleotide esters used in this work.

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LIPASE ACTIVITY IN LINGCOD MUSCLE PREPARATIONS[1]

J. D. Wood

Abstract

A lipase has been isolated and partially purified from the muscle of the lingcod (*Ophiodon elongatus*). Both monoglycerides and triglycerides are hydrolyzed but methyl acetate and ethyl acetate are attacked extremely slowly, if at all. The lipase is inhibited by *p*-chloromercuribenzoate but not by ferricyanide, cyanide, or azide. The pH of optimum activity is 8.2. The enzyme is unstable in aqueous solution, but can be stored at −20° C for several weeks without loss of activity.

Introduction

Frozen fish deteriorates gradually during storage. One aspect of the deterioration is the off-flavor produced by fat spoilage. The most well-known type of fat spoilage is oxidative rancidity. There is, however, a second type, namely, hydrolysis of the fat to produce free fatty acids. Brocklesby (1) showed that there was a gradual increase in free fatty acids during storage of various species of Pacific salmon. Mel'nikova and Khalina (2) observed similar changes in frozen mackerel. More recently Dyer and his co-workers (3, 4, 5) investigated the hydrolysis of lipids in frozen fillets of plaice, halibut, rosefish, and cod which were stored at different temperatures. Fatty acid formation was also observed by Cardin and Bordeleau (6) to occur during the process of salting codfish. At the present moment there are two different theories as to the mechanism by which the fatty acids are formed. Dyer and co-workers believe that the acids are released from lipids by the action of a lipase present in the fish muscle. This view is supported by Cardin and Bordeleau. Lovern *et al.* (7) however favor the theory that the formation of fatty acids is due to bacterial action.

Lipase activity has been studied extensively in material from various sources such as hog pancreas (8), wheat germ (9), and *Fusarium lini* Bolley (10). Several methods have been employed to purify the enzyme including precipitation by ammonium sulphate (9), magnesium sulphate (11), and acetone (10). There are also numerous reports on lipase activity in fish (e.g. 12, 13, 14) but the preparations used were crude and in most cases tissues other than the muscle were employed.

Because of the lack of information on lipase activity in fish muscle and in view of the importance attached to the spoilage of fish during storage, it was decided that an investigation into the occurrence and properties of lipase enzymes in fish muscle would yield useful information. This paper presents the results of such an investigation.

Methods

Lingcod Muscle

The tissue employed in the experiments was obtained from lingcod (*Ophiodon elongatus*) which were obtained fresh. The fish were either used at once or

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Contribution from the Fisheries Research Board of Canada Technological Station, Vancouver, British Columbia.

were frozen and stored at −20° C until used. The enzyme was found to be still active after several weeks at −20° C.

Measurement of Lipase Activity

Two methods were employed for determining the activity. One method was that first proposed by Rona and Lasnitzki (15), which was based on the manometric determination of carbon dioxide evolved from a bicarbonate buffer as a result of the liberation of fatty acids by the enzyme. The other method employed was the titration of the fatty acids liberated.

1. Manometric method. The incubations were carried out in the conventional Warburg apparatus at 25° C and under an atmosphere of 95% nitrogen and 5% carbon dioxide. The total volume of the flask contents was 3.0 ml. The reaction was carried out in 0.025 *M* $NaHCO_3$ unless otherwise stated. This gave a pH of 7.54 when in equilibrium with the gas phase (16). The substrate, dissolved in 0.025 *M* $NaHCO_3$, was placed in the side arm of the vessel. One milliliter of enzyme solution and 1.0 ml 0.05 *M* $NaHCO_3$ were placed in the main compartment. Substrates which were not sufficiently soluble were homogenized in polyvinyl alcohol prepared by the method of Fiore and Nord (17), and which contained 0.025 *M* $NaHCO_3$.

2. Titrimetric method. The technique employed was that reported by Fiore and Nord (17) with the following two alterations. The temperature employed was 25° C and the McIlvaine buffer was replaced by 0.1 *M* tris(hydroxymethyl)aminomethane because of the high pH range (7.8 to 9.0) required during the experiment.

Protein Estimation

This was performed using either the method of Kingsley (18) as adapted by Snow (19) or that of Lowry *et al.* (20).

Isolation and Purification of the Enzyme

A weighed quantity of lingcod muscle, either fresh or frozen, was homogenized in three volumes of ice-cold, distilled water and centrifuged at 20,000 *g* for 20 minutes. The supernatant fluid was collected. In this paper it will be designated muscle extract.

The extract was treated with $MgSO_4$ solution (60 g/100 ml) at 0° C and stirred for 10 minutes. The mixture was centrifuged at 20,000 *g* for 20 minutes and the protein precipitate was resuspended in ice-cold, distilled water and made up to a volume equal to one-fourth of that of the original extract. The suspension

TABLE I

Purification of lingcod muscle lipase

Preparation	μl CO_2 evolved/ hour/mg protein N	Activity recovered (%)	Purification
Muscle extract	91	100	
Precipitated with $MgSO_4$	945	18	10.4
Precipitated twice with $MgSO_4$	912	5	10.0

NOTE: Substrate was 8.4×10^{-2} *M* triacetin.

was filtered through glass wool and the filtrate collected. This preparation was employed in the experiments detailed in this paper unless otherwise stated.

The purification obtained by this method was variable within the range fivefold to tenfold. Table I shows the results of a typical purification of the enzyme. The activity recovered was low but this was not too important since only one fractionation was employed in the routine procedure. A second precipitation with $MgSO_4$ resulted in no further purification, but only in a decreased yield of enzyme.

Results

The enzyme preparation was found to be somewhat unstable in aqueous solution. There was a significant loss in activity when the preparation was dialyzed overnight at 0° C. Table II shows the effects of dialysis on the ability

TABLE II

Stability of lingcod muscle lipase

Preparation	μl CO_2 evolved/flask/hour in expts.:				
	1	2	3	4	5
Non-dialyzed	83	119	69	76	155
Dialyzed against 0.01 *M* $MgCl_2$	71	34		74	
Dialyzed against H_2O	42	14	47	45	75
Dialyzed against H_2O, $MgCl_2$ added to give concentration of 0.01 *M*			43	47	
Dialyzed against 0.01 *M* cysteine					75

NOTE: Substrate was 8.4×10^{-2} *M* triacetin. Dialysis overnight except expt. 2, which was for 72 hours.

of the enzyme to hydrolyze triacetin. The loss in activity with dialysis was reduced but not eliminated by dialyzing against 0.01 *M* $MgCl_2$. The addition of $MgCl_2$ to the enzyme preparation which had been dialyzed against water did not restore the loss in activity. The presence of cysteine during dialysis failed to prevent the destruction of the enzyme. The preparation was stable in the frozen state and could be kept at −20° C for 4 weeks without any loss of activity.

The lipolytic activity of the enzyme preparation was studied at a series of pH's from 6.34 to 7.84 using the manometric technique. The results (Fig. 1) show that the reaction rate increased with pH throughout the entire range. Studies at higher pH's could not be carried out by this method due to the limitations imposed by the bicarbonate–CO_2 buffer system. The higher pH range was studied using the titrimetric method. The results obtained are shown in Fig. 1. The pH of optimum activity was 8.2. At higher pH values the activity decreased sharply.

The hydrolysis of triacetin was studied at temperatures varying from 5° C to 45° C and the results are shown in Fig. 2. The rate of hydrolysis increased with temperature over the whole range employed. However, at 45° C and to a slight extent at 35° C a decrease in the reaction rate during the course of the 1 hour incubation was observed. No such loss in activity occurred at 5, 15, or

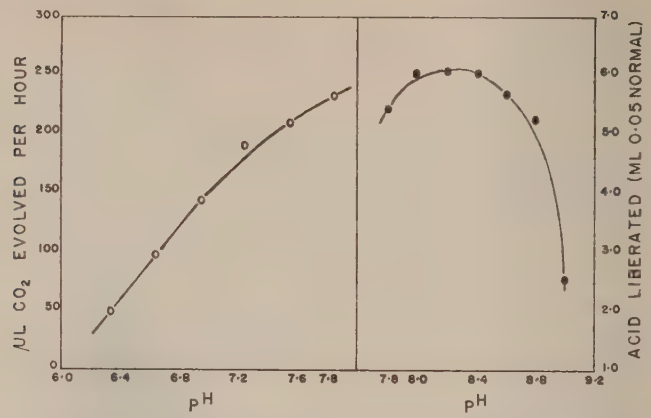

FIG. 1. Effect of pH on the rate of hydrolysis of triacetin by lingcod muscle lipase. Substrate concentration was 8.4×10^{-2} *M*, and 2.5×10^{-2} *M* for the manometric and titrimetric estimations respectively. The incubation for the latter method was 4 hours.

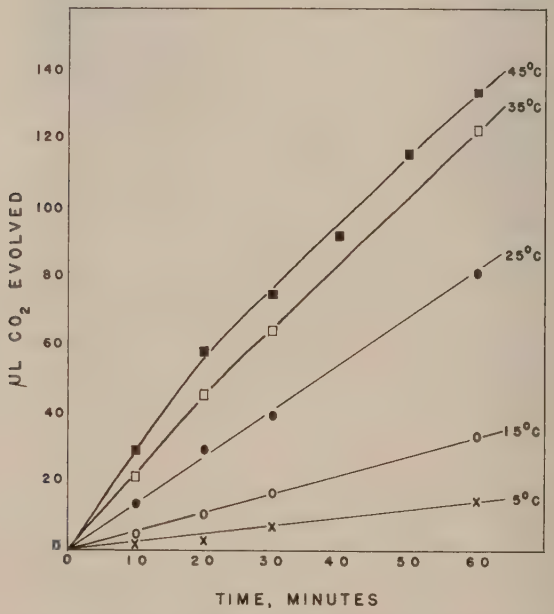

FIG. 2. Effect of temperature on the rate of hydrolysis of triacetin by lingcod muscle lipase. Substrate concentration was 8.4×10^{-2} *M*.

25° C. Moreover, the rate of hydrolysis at 45° C decreased when the amount hydrolyzed was less than that hydrolyzed after 1 hour at 25° C, at which time the reaction rate was still constant at the lower temperature. This indicates that the loss in activity at the higher temperatures was due to a destruction of the enzyme rather than to a shortage of substrate caused by the more rapid activity.

The effect of the lingcod muscle lipase on different substrates was studied (Table III). Both monoglycerides and triglycerides were attacked but methyl

TABLE III

Relative rates of hydrolysis of various esters by lingcod muscle lipase

Substrate	Conc.	Relative rate of hydrolysis (8.4×10^{-2} M triacetin = 500)
Triacetin	8.4×10^{-2} M	500
Tripropionin*	8.4×10^{-2} M	490
Tributyrin*	8.4×10^{-2} M	242
Trioctanoin*	8.4×10^{-2} M	2
Monoacetin	8.4×10^{-2} M	151
Monopropionin	8.4×10^{-2} M	233
Monobutyrin	8.4×10^{-2} M	295
Tween 20	5%	166
Methyl acetate	8.4×10^{-2} M	8
Ethyl acetate	8.4×10^{-2} M	13
Olive oil*	10%	3
Halibut liver oil*	10%	0
Lingcod liver oil*	10%	0

*Insoluble or not completely soluble in the concentrations employed and therefore added as a suspension.

acetate and ethyl acetate were hydrolyzed extremely slowly, if at all. The triglycerides containing longer chain fatty acids such as trioctanoin were not attacked. Olive oil, which is often used as a substrate in lipase studies, was not hydrolyzed by the fish muscle enzyme even when the oil was present as a very fine emulsion. Halibut liver oil and lingcod liver oil were likewise inactive. However, the enzyme was capable of hydrolyzing long chain fatty acids, as is shown by its action on Tween 20 which is the water-soluble monolaurate ester of a polyalkylene derivative of sorbitol. The results in Table III show that the rate of hydrolysis of the monoglycerides increased with the chain length of the fatty acid. The same increase in activity with chain length did not apply to the triglycerides. However, triacetin was soluble at the concentrations used whereas tripropionin and tributyrin were not completely soluble. In spite of this relative insolubility, tripropionin proved just as reactive as triacetin, while tributyrin showed half the activity. Triglycerides appeared

TABLE IV

The effect of various compounds on the hydrolysis of triacetin by lingcod muscle lipase

Compound	Conc., M	% control
p-Chloromercuribenzoate	1×10^{-3}	86
Potassium ferricyanide	2×10^{-3}	99
Sodium iodoacetate	2×10^{-3}	98
Sodium iodoacetate	1×10^{-2}	95
Potassium cyanide	1×10^{-5}	97
Potassium cyanide	1×10^{-3}	97
Potassium cyanide	1×10^{-2}	100
Sodium azide	1×10^{-3}	98
Sodium azide	1×10^{-2}	103

NOTE: Substrate concentration was 8.4×10^{-2} M.

to be more susceptible to attack than monoglycerides because triacetin was hydrolyzed more rapidly than monoacetin.

The effect of some common inhibitors of enzyme action on triacetin hydrolysis is shown in Table IV. Cyanide and azide in various concentrations had little or no effect on the activity. Of the sulphydryl reagents that were tested, *p*-chloromercuribenzoate and possibly iodacetate brought about inhibition of the lipase activity. However, the inhibition was never great even with the *p*-chloromercuribenzoate.

Discussion

The results presented here show that the lingcod muscle lipase was rather unstable in aqueous solution especially if no inorganic salts were present. This instability in solution is frequently encountered in lipase studies. Fiore and Nord (17) reported similar findings with *Fusarium lini* Bolley lipase, and they claimed that it was due to destruction of the enzyme by proteolysis. Singer and Hofstee (9), investigating wheat germ lipase, observed a loss in activity during overnight storage at 3° C. These workers, however, were of the opinion that the inactivation was due to oxidation of essential SH groups since the inactivation was reduced by the presence of glutathione. The destruction of lingcod muscle lipase does not appear to be due to SH oxidation because cysteine did not prevent the destruction of the enzyme. Moreover, sulphydryl poisons did not have a very marked effect on the activity. This is to be compared with the results obtained by Singer and Hofstee for their wheat germ lipase. Under similar conditions to those used in the present experiments, the wheat germ lipase was inhibited 87% by *p*-chloromercuribenzoate and 43% ferricyanide. The inhibition of the lingcod muscle enzyme by these compounds was 14% and 1% respectively.

The lingcod muscle lipase attacks both monoglycerides and triglycerides but has little effect on methyl acetate and ethyl acetate. This indicates that the enzyme is a lipase rather than an esterase acting on simple esters of low molecular weight acids. However, the enzyme is without action on trioctanoin and olive oil. This at first sight would indicate that the fish muscle enzyme does not hydrolyze molecules containing longer chain fatty acids. However, it was observed that lauric acid was split from the water-soluble Tween 20. In addition, the activity of the soluble monoglycerides increased with the chain length of the acid. The possibility therefore arises that it is not so much the chain length of the glyceride which is preventing attack but rather the insolubility of the compound.

The present investigation has shown that an enzyme exists in lingcod muscle which can hydrolyze lipid material. The possibility that the formation of free fatty acids during storage of fish fillets is due to enzymes in the muscle cannot therefore be ignored. At the temperatures employed for the storage of fish (−10° C to −20° C) the enzyme would of course have to act in firmly frozen material. Balls and Tucker (21) have shown that lipase enzymes can indeed act at low temperatures. They reported that an enzyme system from dried pig

pancreas was able to hydrolyze olive oil and tributyrin at temperatures as low as −25° C.

The results reported in this paper have concerned the action of a lipase which may possibly be acting on fish muscle to liberate free fatty acids. The phospholipase group of enzymes, however, cannot be ignored. The phospholipids are major components of the lipids in some species of fish. The lingcod belongs to this group and the release of fatty acids may be due to the action of phospholipases rather than lipases. An investigation is at present being conducted into the occurrence of phospholipases in fish muscle. The possibility that the hydrolysis is due to non-enzymic catalysis must, of course, always be borne in mind.

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LINGCOD MUSCLE PHOSPHORIBOISOMERASE AND RIBULOSE 5′-PHOSPHATE 3′-EPIMERASE[1,*]

H. L. A. Tarr

Abstract

Comparatively pure phosphoriboisomerase and ribulose 5′-phosphate 3′-epimerase enzyme preparations were obtained from lingcod muscle by a simple procedure involving water extraction, saturation of the extract with ammonium sulphate, dialysis, brief heating to 55° C, lyophilization of the solution, and final separation by ion exchange chromatography, using diethylaminoethyl cellulose columns. Both enzymes have broad pH optima above pH 7.0, but are rapidly inactivated below this value. The following equilibria were established and compared with those obtained by other investigators: R5P ⇄ RU5P, 1.35:1.0; RU5P ⇄ XU5P, 1:1.5 and R5P ⇄ RU5P ⇄ XU5P, 1:0.58:0.66. The ketopentulose phosphate resulting from the action of phosphoriboisomerase on D-ribose 5-phosphate was isolated and identified as D-ribulose 5-phosphate. Both D-ribulose and D-xylulose were demonstrated after subjecting a product of epimerase action to hydrolysis by acid phosphatase and ion exchange chromatography.

Previous investigations have been concerned with the nucleoside hydrolase and phosphorylase enzymes of lingcod (*Ophiodon elongatus*) muscle, and with a crude phosphoriboisomerase enzyme preparation from this source by the action of which deoxyribose 1,5-diphosphate was prepared for the first time (1).

The importance of pentose phosphate esters in biochemical processes has become increasingly apparent in recent years. Thus, oxidative decarboxylation of glucose 6-phosphate via the so-called hexose monophosphate shunt pathway leads to formation of RU5P (2). Two enzymes are required to transform this compound into substrates for transketolase. These are epimerase, which catalyzes formation of XU5P, the donor, and PRI, which catalyzes formation of R5P, the acceptor aldehyde. The present paper describes the isolation, partial purification, and some of the properties of these enzymes as obtained from lingcod muscle.

Materials

o-Nitrophenylhydrazine used in preparation of ribulose *o*-nitrophenylhydrazone was made from *o*-nitroaniline (3). D-Ribulose *o*-nitrophenylhydrazone was prepared by the method of Glattharr and Reichstein (4) and recrystallized from absolute methanol. The melting point and specific rotation agreed well with those obtained by other investigators: $[\alpha]_D^{21}$ +51.8° in methanol (*c*, 1.35); m.p. 165.5–166° C (tube); 163–164° C (block) (3, 4). D-Xylulose *p*-bromophenylhydrazone was prepared as described by Schmidt and Treiber (5). This

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*The following abbreviations are used: R5P, D-ribose 5-phosphate; RU5P, D-ribulose 5-phosphate; XU5P, D-xylulose 5-phosphate; PRI, phosphoriboisomerase; EPIM, ribulose 5′-phosphate 3′-epimerase; Tris, tris-hydroxymethylaminomethane; DEAE, diethylaminoethylcellulose; XU, xylulose; RU, ribulose.

compound (m.p. 128–128.5° C) exhibited the pronounced mutarotation noted by the above workers: $[\alpha]_D^{21}$ +19.9°–20.6° 15–20 minutes after dissolving in anhydrous pyridine; (*c*, 2.094) and $[\alpha]_D^{2}$ −34.2° to −35.6° after 7–8 days at this temperature. These compounds were used directly as standards in the cysteine–carbazole reaction (6), and were decomposed as described by Schmidt and Treiber (5) to obtain the free pentuloses for chromatographic standards.

Barium R5P was prepared from chromatographically pure 5′-adenylic acid (Schwarz) and was dried in high vacuum over P_2O_5 (7). This compound was practically free from orthophosphate, possessed about the theoretical organic phosphorus and pentose content (based on mol. wt. 365.5), and gave the specific rotation characteristic of R5P of high purity (2); $[\alpha]_D^{23}$ +23.2° in 0.2 *N* HCl (*c*, 1.909) based on the free acid, mol. wt. 230). The RU5P content was 2–3% as determined by the cysteine–carbazole reaction and the presence of alkali-labile pentose. The enzymic preparation of RU5P is described later.

DEAE cellulose Type 20 was obtained from the Brown Co., Berlin, New Hampshire. Dowex 1×10 and Dowex 50×8 (200–400 mesh, analytical grades) were obtained from the California Foundation for Biochemical Research, and Amberlite IR 45 (analytical grade) from the Fisher Scientific Co.

Methods

Aldopentose and aldopentose phosphates were determined by the orcinol procedure (8) with a 40-minute heating period and arabinose as standard. The optical density at 670 mμ with pentulose phosphates in this method was multiplied by 1.76 in order to determine the concentration present, since these compounds give about 57% of the value of an identical amount of arabinose on the basis of the pentulose content (9, 10). With this method the average ratios of absorbancy at 540/670 mμ were: D(−)ribose, 0.21; D(−) and L(+)arabinose, 0.19; D(−)ribulose, 0.85; D(−)xylulose, 0.49; and D-RU5P, 0.33.

Alkali-labile pentulose was determined by adjusting the sample to 1.0 *N* with 2 *N* NaOH, holding 20 minutes at 25°, and then carrying out the orcinol reaction (11). Under these conditions pentuloses appear to be completely inactivated while R5P is very resistant. Tests indicated that approximately 4% of R5P is inactivated during this treatment, and this was taken into consideration when the method was used.

Pentuloses and pentulose phosphates were also determined directly by the cysteine–carbazole reaction (6) after 15 minutes for ribulose and 120 minutes at 30° for xylulose. The proportion of ribulose and xylulose in mixtures containing between 10 and 30 μg of the pentuloses was determined by calculating the ratio of the optical density at 540 mμ in the cysteine–carbazole reaction after 15 minutes and after 120 minutes. In this test the ratio with ribulose alone was 1.04 and with xylulose alone 0.55. This method required prior hydrolysis of the pentulose phosphates with acid phosphatase (see below) but proved more sensitive than that of Ashwell and Hickman (12), in which the proportion of pentulose phosphates is determined directly using a modified cysteine–carbazole reaction. Pentulose phosphates were determined directly using the cysteine–

carbazole reaction with a 2-hour heating period at 37°. Under these conditions experience showed that the amount of pentulose found multiplied by the factor 1.85 gave the concentration of pentulose in pentulose phosphate.

Protein was determined by the quantitative biuret method (13) or by that of Lowry *et al.* (14), the latter proving exceedingly useful where only small amounts of protein were measured. The method of Lowry and Lopez (15) with the modification of Bruemmer and O'Dell (16), and also that of Gomori (17) were used to determine orthophosphate, after appropriate hydrolysis procedures where necessary.

Acid phosphatase was prepared from "Polidase" (Schwarz), using a recent modification (9) of the original procedure of Hochster (18). Its activity (226 μmoles of R5P hydrolyzed by 1.0 ml in 30 minutes at 37°) was similar to that reported by the above investigators.

Barium ions were removed from Ba R5P and Ba RU5P by passage through small columns of Dowex 50 H^+ resin, adjusting the effluent and washings to pH 7–7.5 with NaOH or KOH as required, and standardizing the solutions by the appropriate methods.

5-Phosphoribonic acid was prepared by oxidation of Ba R5P with alkaline periodate (19) to yield the tricalcium salt $(C_5H_8O_9P)_2Ca_3$ (20) (P calc. 10.23; found 9.77).

All enzyme reactions were carried out at 37° C. Chromatographic separation of the R5P and RU5P was carried out using 16-hour descending development on Whatman No. 1 paper with *n*-propanol:28% NH_4OH:H_2O (6:3:1). Separation of the free pentuloses was effected by 16-hour ascending development, using 90% phenol as solvent in presence of KCN crystals. The usual spray reagents were employed (9, 18, 21). The enzyme reactions were carried out in 160×15 mm tubes, the appropriate colorimetric reaction being carried out in the same tube.

Experimental

Preparation of Enzymes

In this work one unit of enzyme is arbitrarily defined as that amount which caused turnover of 1 micromole of substrate in 1 minute at 37° C where conditions were such that the reaction was approximately linear with respect to time (22). The specific activity is recorded as units per milligram of protein.

Satisfactory preparations were obtained from fresh or frozen lingcod, and all procedures were carried out at 0 – 3° C. In a typical case 1.5 kg of the muscle of a fish which had been frozen and stored about 3 months at −20° C was thawed until it could be cut readily, and was then blended for about 1 minute with 3 liters of water and sufficient 1 *N* HCl to adjust the suspension to pH 6.0. The suspension was centrifuged 15 minutes at 14,000 g. The supernatant liquid was filtered through glass wool to yield 1460 ml of fairly clear liquid. This was saturated with $(NH_4)_2SO_4$ (2.46 kg) at pH 7.0, about 35 ml of 2 *N* NaOH being required to adjust the reaction. The precipitated protein was collected by filtering for 16 hours at 0° C through Whatman No. 1 paper. The moist filter cake (about 250 g) was dialyzed 2 hours against running tap

water (7° C) and then for 24 hours against several changes of demineralized water to yield 870 ml of crude enzyme preparation (16.8 mg of protein/ml) (Table I).

TABLE I

Purification of PRI

Fraction	Ml	Mg protein per ml	Total protein, mg	Specific activity	Total units
Saturated $(NH_4)_2SO_4$	870	16.8	14,700	0.175	2,565
Heated to 55° C and lyophilized	179	39.4	7,050	0.284	2,000
Eluate from DEAE column	4,350	0.015	65	20.4	1,350

Crude enzyme (170 ml), in a stainless steel tube immersed in a 65° C water bath, was stirred rapidly until the temperature attained 55° C, and was then promptly chilled to 0° C. The coagulated protein was removed by centrifuging 5 minutes at 10,000 *g*. The clear supernatant liquid was lyophilized and the residue suspended in water, insoluble protein being removed by centrifuging. The pH, which was about 5.5, was cautiously adjusted to pH 7.0 with a little 1 *N* NaOH to yield 35 ml of a solution containing 39.4 mg protein/ml.

A jacketed column containing 13 g dry weight of DEAE (23) (34×1.8 (diam.) cm) was conditioned with 0.02 *M* Tris–HCl buffer, pH 7.0, and 6 ml (236 mg protein) of the lyophilized enzyme preparation in 0.02 *M* Tris was run onto the column, which was maintained at 1° C. The column was eluted at 1.3 ml/minute under 5 lb/sq. in. pressure, using a gradient in which 0.25 *M* Tris–HCl buffer was run into 250 ml of 0.02 *M* Tris–HCl, 5 ml fractions being collected. The chilled fractions were tested for protein and for PRI and EPIM activity. These eluates were conveniently buffered at pH 7–7.3 so could be assayed directly.

The position of PRI was determined by incubating 0.1 ml of the column eluates directly with 0.02 ml of a 10 μmole/ml solution of Na R5P, and determining the RU concentration by the 2-hour cysteine–carbazole procedure. The position of EPIM was determined by three different procedures as follows: (1) 0.04 ml of eluate was incubated with 0.02 ml of Na RU5P solution (0.2 μmole) at pH about 7.2 for 30 minutes at 37° C. The solutions were promptly chilled to 0°, 0.01 ml of 0.004% methyl red indicator, 0.01 ml of 0.01 *M* $MgCl_2$, and sufficient 0.1 *M* CH_3COOH to adjust the pH to about 5.0 (0.01–0.015 ml) were then added. After addition of 0.005 ml of acid phosphatase to each, the tubes were again incubated as above and the proportions of xylulose and ribulose determined by the cysteine–carbazole method. (2) One-tenth milliliter each of the column eluate and a 10 μmole/ml solution of NaRU5P were incubated 10 minutes at 37° C and a modified cysteine–carbazole reaction (12) carried out directly, the proportions of RU5P and XU5P being determined by the change in optical density due to XU5P formation. (3) One-tenth milliliter each of eluate and a NaRU5P solution (2.5 μmoles) were incubated 1 hour at 37° C. Acid phosphatase (0.1 ml), 0.1 *M* $MgCl_2$ (0.01 ml), and 0.1 *M* acetic acid to adjust the pH to about 5.0 with methyl red indicator were added and

the solutions incubated 1 hour at 37° C. They were concentrated to about 0.1 ml *in vacuo* over P_2O_5, a little Dowex 50 H^+ resin was added to remove cations, and the solution chromatographed using 90% phenol. After the chromatograms were dried and residual phenol extracted with diethyl ether RU (R_f 0.65) and XU (R_f 0.55) were detected by the trichloracetic acid – orcinol spray (21), followed by aniline phosphate (24) or aniline hydrogen phthalate (18) sprays.

The results obtained in this experiment are shown in Fig. 1. With PRI the results obtained with a second identical column are also shown to indicate the

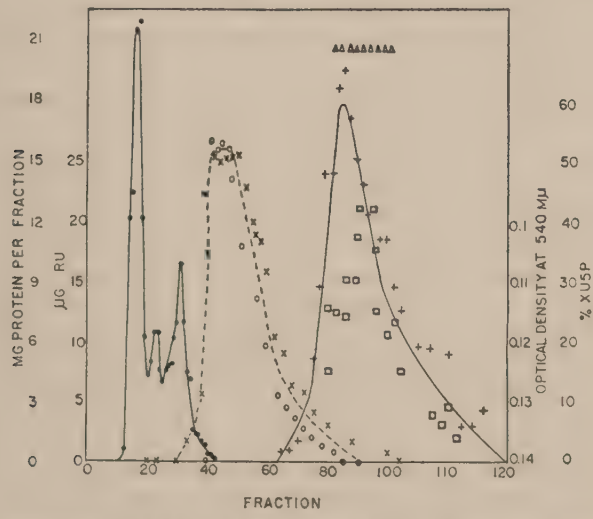

FIG. 1. Separation of PRI and EPIM using a DEAE column. Protein ●——●. PRI as determined by RU concentration (two different columns) ×- - -× and ○- - -○. EPIM as determined by % XU5P formed +—— +; by decrease in O.D. at 540 mμ after 30 minutes at 37° C in the modified cysteine–carbazole reaction and recalculated on the basis of 0.1 μmole of RU5P giving O.D. of 0.15 and 0.1 μmole of XU5P, O.D. of 0.05 under these conditions (12) □——□; and by paper chromatography method as indicator of XU △ △ △.

close reproducibility obtained. No significant PRI or EPIM activity was found in the three major protein peaks emerging up to fraction 35, and these fractions accounted for 204 mg, or 86%, of the protein applied to the column.

PRI was obtained by pooling fractions 40–70 inclusive (146 ml). The preparation had 15 μg of protein per ml and a specific activity of 20.4 (Table I). If it is assumed that the original muscle (1.5 kg) had 2560 units of PRI, and the muscle protein content was 18%, then the specific activity would be roughly 0.01. On the basis of the original muscle the maximum purification attained in the procedures as outlined in Table I would then be about 2000 times.

Fractions 78–110 inclusive (Fig. 1) were pooled to yield 156 ml of EPIM preparation containing 5 μg of protein per ml. Since the purified enzyme is rather labile, all tests were carried out within 1 week at 0° C. A number of tubes containing 0.05 ml of EPIM plus 2 μmoles of NaRU5P in 0.02 ml were

incubated at 37° C and four tubes removed at intervals of 5, 10, 15, and 20 minutes for determination of XU5P formation by the cysteine–carbazole reaction after hydrolysis by acid phosphatase. The results showed that under these conditions 0.25 μg of protein (per sample) formed 0.0054 μmole of XU5P in 1 minute. Thus this preparation had a specific activity of 21.6. The recovery from 1.5 kg of muscle was 233 units as compared with 1330 units of PRI. While PRI proved very stable when frozen, EPIM preparations lost all measurable activity within 3 months at about −20° C.

Alternative Method for Preparation of PRI

The above DEAE column method provided a fairly satisfactory separation of PRI and EPIM. An alternative rapid procedure for preparing PRI free from EPIM is as follows:

In a typical experiment 43 ml of crude ammonium sulphate fraction containing 18.8 mg of protein per ml was heated to 55° and chilled as described above and centrifuged. The supernatant liquid was fractionated with $(NH_4)_2SO_4$ at pH 7.0, and the fraction precipitating between 0.6 and 0.75 saturation collected and dialyzed against frequent changes of demineralized water for 24 hours at 0° C to yield 8 ml of a preparation containing 42.5 mg/ml of protein. This preparation possessed a specific activity of 1.27, but the actual recovery was poor since much of the PRI remained in the fraction precipitating below 0.6 saturation with $(NH_4)_2SO_4$. This preparation was free from EPIM as determined by incubating samples with RU5P, dephosphorylating with phosphatase, and carrying out chromatography for RU and XU as described above. Only RU was found.

Preparation of RU5P

The barium was removed from BaR5P (2.6 mmoles) as described under Methods, and a solution of the potassium salt was obtained and incubated at pH 7.2 with 6.0 ml (7.6 units) of the PRI described in the preceding paragraph. The amount of RU5P formed was determined at intervals using the cysteine–carbazole reaction and the 1.85 factor. After 2.5 hours about 33% of the R5P was isomerized and, after a further 2 hours the reaction was complete with 43% RU5P formation. The solution was chilled to 0° C, deproteinized with 3 ml of 60% perchloric acid, and centrifuged 5 minutes at 10,000 *g*. The clear supernatant liquid was adjusted to pH 6.0 with 1 *N* KOH and the solution evaporated to dryness at 40° using a rotary evaporator. The dry residue was extracted at 0° C with water, the potassium perchlorate being removed by filtration. The clear filtrate and washings (35 ml) were treated with bromine water to remove aldopentose phosphate (24).

The clear colorless filtrate was run slowly onto a 12×2.8 (diam.) cm Dowex 1×10 formate column, washed with 100 ml of H_2O, and eluted with 0.1 *M* sodium formate in 0.1 *M* formic acid at 15 ml per minute, 14-ml fractions being collected. Fractions 41–56 inclusive, which contained practically all the cysteine–carbazole-positive material were pooled. The solution was adjusted to pH 5.0 with 1 *N* KOH, and then to pH 6.2 with warm, freshly filtered barium hydroxide solution. The BaRU5P was precipitated by adding four volumes of

absolute ethanol and the flocculent precipitate collected by centrifuging. The precipitate was dissolved in 50 ml of H_2O, a small amount of insoluble material removed by centrifuging, and the BaRU5P reprecipitated and dried in high vacuum over P_2O_5 at 0°. The yield was 250 mg of approximately 78% pure BaRU5P as judged by the cysteine–carbazole and orcinol reactions.

The product was dissolved in 3 ml of H_2O and run slowly through a 3×2 cm Dowex 50×8 H^+ column at about 3° C. The eluate and washings, after removal of insoluble material by centrifuging, were promptly adjusted to pH 6.2 with warm barium hydroxide solution, the BaRU5P precipitated and collected, and washed with 80% and absolute ethanol before being dried in high vacuum in P_2O_5 at 0° (yield 170 mg). The product gave 55.5 to 59% of the absorption of arabinose at 670 mμ in the orcinol reaction, 51% of the absorption of free RU after 2 hours at 37° C in the cysteine–carbazole reaction, was free from orthophosphate, and had 97% of the theoretical organic P content (assuming mol. wt. 365.5). It contained 7.0% alkali-stable pentose phosphate, the ratio of optical density 540/670 mμ was 0.33 in the orcinol reaction, and the specific rotation was $[\alpha]_D^{22}$ −20.2° (c=1.85 in 0.2 N HCl) based on the free acid (mol. wt. 230). This rotation compares favorably with that given by a fairly pure sample of L-RU5P; $[\alpha]_D^{20}$ +20° (25). Two zones, one characteristic of RU5P (2.5–3.0 cm from origin) and the other of R5P (6.0 cm from origin) resulted when the free acid was chromatographed. On the basis of these data the product contained about 90% BaRU5P and 7.0% R5P. This, or products of similar purity, were used in the following studies.

pH-Activity of PRI

Portions of a solution containing 10 μmole/ml of NaRU5P were either used without addition or were made 0.05 M with respect to glycine or sodium acetate. These were then adjusted to different pH values by cautious addition of traces of 1 N HCl or 10 N NaOH (Fig. 2). Duplicate 0.1-ml portions of these solutions were incubated 10 minutes with 0.02 ml of purified PRI (1 μg of protein) and the amount of RU5P formed was determined by the 2-hour cysteine–carbazole reaction and expressed as micrograms of RU. The results (Fig. 2) show that the enzyme has an optimum activity between about pH 7 and 8, loses its activity markedly between about pH 7 and 4, but has considerable activity at pH values between 8 and 11. A rather similar pH-activity pattern was observed for rabbit muscle PRI (24).

pH-Activity of EPIM

Purified EPIM containing 5 μg/ml of protein, and buffered with approximately 0.2 M Tris-HCl pH 7.3 as eluted from a DEAE column, was adjusted to lower pH values with very small amounts of 0.1 M acetic acid and to higher pH values with 1 N NaOH. Tubes containing 0.05 ml of buffered enzyme and 0.02 ml (0.19 μmole) of NaRU5P solution were incubated at 37° for 30 minutes. They were promptly chilled to 0° C and subjected to hydrolysis with acid phosphatase for 30 minutes at 37° under similar conditions to those described in the section dealing with isolation of the enzyme. The concentration of RU5P formed was determined by the cysteine–carbazole reaction (Method 1). The

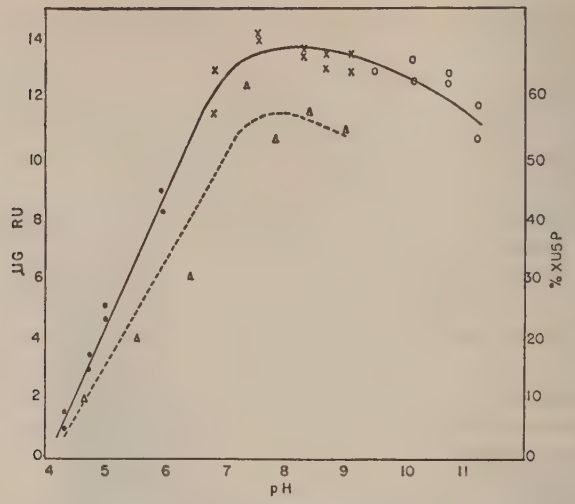

FIG. 2. pH–activity curves for PRI and EPIM. PRI with acetate ●——●, Tris ×——×, and glycine–NaOH ○——○ buffers. EPIM △- - -△ (see text for method used in adjusting pH).

results, shown in Fig. 2, indicate that the pH–activity curve of this enzyme is quite similar to that of PRI.

Equilibrium with Crude Preparations Containing both PRI and EPIM

Two preparations were studied, namely, the supernatant liquid from a crude enzyme which had been heated to 55° C and centrifuged (10.4 mg per ml of protein) and a similar preparation which had been lyophilized (35 mg per ml of protein). Duplicate portions of these preparations (0.01, 0.02, and 0.04 ml) were incubated at pH 7.2 for 10 minutes at 37° with 0.02 ml of a solution containing 0.18 μmole of NaR5P. The reaction was promptly stopped by immersing the tubes in a solid CO_2–acetone mixture, and the samples were subjected to analysis for total pentulose phosphate and XU5P content by the cysteine–carbazole method after prior hydrolysis with acid phosphatase. Similar results were obtained with the two enzyme preparations used and with the three concentrations. The total pentulose phosphate concentration varied between 50% and 58.5% (average 53.5%) of the initial amount of R5P used. The XU5P concentration varied between 52% and 59% (average 54%) of the total pentulose phosphate, and that of the RU5P between 41% and 48% (average 46%). The average R5P content (by difference) was 46.5%. The proportion of phosphate esters in this experiment, therefore, was about R5P, 46%; XU5P, 29%; RU5P, 25%.

A similar experiment was carried out using only 0.01 and 0.02 ml of the same two enzyme preparations and half the amount of R5P (0.095 μmole). In this instance the concentration of alkali-labile pentulose phosphate was determined. When the reaction was complete the samples contained from 54.5% to 56.6% (average 55.7%) of alkali-labile pentulose phosphate. In this

experiment the ratio was therefore: total pentulose phosphates, 55.7%:R5P, 44.3%, which is similar to that found in the preceding test.

Equilibrium with a Mixture of Purified PRI and EPIM

A mixture containing 19 μmoles of NaR5P, 2 ml of purified PRI (0.03 units), and 6 ml of EPIM (1.4 units) in a 10-ml volume was incubated at pH 7.2 and 37° C, duplicate 0.5-ml samples being removed at 10-, 30-, 45-, 60-, and 90-minute intervals for cysteine–carbazole determinations after dephosphorylation with acid phosphatase in the usual manner. The results showed that the total pentulose phosphate concentration increased up to 45 minutes, and that thereafter it remained approximately constant. The XU5P:RU5P ratio did not alter appreciably throughout the experiment, and the results obtained after 60 and 90 minutes showed that this ratio was 57% XU5P:43% RU5P, and that the total pentulose recovered was 1440 μg.

Alkali-stable pentose phosphate was also determined, using duplicate 0.15- and 0.20-ml aliquots of the reaction mixture after the 90-minute period. It was found that between 1080 and 1150 μg (av. 1110 μg) of alkali-stable pentose remained. Thus, the total recovery was 2550 μg of pentose plus pentulose, or 90% of the pentose of the R5P used. The ratio calculated from the above data was 43.0% R5P:32.5% XU5P:24.5% RU5P, which agrees well with that obtained with the crude mixed enzyme preparation in the preceding experiment.

Equilibrium with Purified PRI

Tubes containing 0.095 μmole of NaRU5P and 0.05 ml of a purified PRI preparation (1.2 μg of protein) were incubated at 37° C and the decrease in RU5P content was measured at intervals in one series by the cysteine–carbazole reaction and in another series by decrease in alkali-labile pentulose phosphate. A similar experiment was made except that NaR5P was used instead of RU5P. The results (Fig. 3) show that equilibrium was attained with 43–45% RU5P: 55–57% R5P.

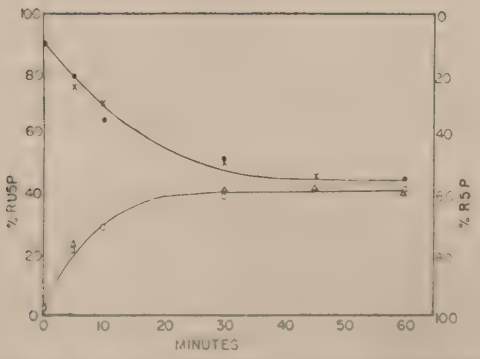

FIG. 3. Equilibrium with purified PRI. Enzyme plus RU5P, R5P formation determined by the cysteine carbazole reaction ●——● and by increase in alkali-stable pentose ×——×. Enzyme plus R5P, RU5P formation determined by the cysteine carbazole reaction ○——○ and by decrease in alkali-stable pentose △——△.

In a second experiment 0.5 mmole of NaR5P was incubated at 37° and pH 7.4 with 85 ml of PRI (2 mg protein = 41 units) in a total volume of 100 ml. To ensure equilibrium the solution was incubated 40 minutes. Analyses then showed 43.6% RU5P by measurement of alkali-labile pentulose, and 42.1% by the cysteine–carbazole reaction. The total pentose content by the orcinol reaction indicated a decrease of 22.5% at 670 mμ. This would account for the formation of 40% RU5P which gives about 57% of the optical density of R5P in this reaction. The average of these determinations indicated that the solution contained 42% RU5P and 58% R5P.

The solution was deproteinized at 5° C with 2 ml of 60% perchloric acid, centrifuged 5 minutes at 10,000 *g*, and the clear supernatant liquid adjusted to pH 5.5 with 1 *N* KOH. The solution was evaporated to dryness using a rotary evaporator at 37° C. The potassium perchlorate was largely removed from the crystalline residue by several extractions with water (1° C) followed by filtration. After adjusting the solution to pH 7.2 it was run onto a jacketed Dowex 1×10 formate column 35×1.8 (diam.) cm, and was eluted at 1° C at the rate of 3 ml/minute, first with 0.15 *M* ammonium formate containing 0.0005 *M* sodium borate, and then with 0.5 *M* sodium formate (24). The RU5P was removed with the first eluent (fractions 72–107) and the R5P (fractions 113–128) with the second eluent. The RU5P fraction (460 ml with washings) accounted for 34.4% of the R5P used, as determined by the cysteine–carbazole reaction using the 1.85 factor, and 35.4% as determined by optical density at 670 mμ in the orcinol reaction using the 1.76 factor. The second fraction (205 ml with washings) contained 49% of the R5P used as determined by the orcinol reaction. The recovery was therefore 84% of the R5P used and the ratio of RU5P:R5P was 1:1.5. Since further elution of the column yielded no material which gave positive cysteine–carbazole or orcinol reactions, it would appear that no significant amount of 3-ketopentulose 5-phosphate (12, 24) or xylose had formed.

A portion of each of the fractions was evaporated to a small volume at low temperature using a rotary evaporator, and the concentrates, after treatment with a little Dowex 50 H^+ resin to remove cations were chromatographed, using the *n*-propanol:ammonium hydroxide:water system referred to previously. Only single zones, characteristic of R5P and RU5P were found. The ratio of optical density at 540/670 mμ of the RU5P fraction in the orcinol reaction averaged 0.334, and that of the R5P fraction 0.19.

Equilibrium in a Crude Enzyme Preparation and Separation of Free X U and R U

Three millimoles of NaR5P were incubated with 15 ml of a lyophilized crude enzyme preparation (59 mg protein) in 250 ml total volume at pH 7.2. Analyses of duplicate 0.05-ml aliquots by the cysteine–carbazole method showed that the reaction neared completion in 20 minutes and was complete in 30 minutes with formation of 1.56 mmoles of pentulose phosphates (52% of the ribose in R5P used). The solution was deproteinized with perchloric acid and potassium perchlorate removed as in the preceding experiment, the R5P being removed by bromine treatment (24).

The clear solution (8.7 ml) was adjusted to pH 5.2 with 1 *N* KOH, and 0.8 ml of 1 *M* $MgCl_2$ and 5 ml of acid phosphatase were added. Testing of 0.02-ml aliquots at intervals showed that formation of orthophosphate (15) became constant after 2.5 hours, but that only 84% of the pentulose phosphates had apparently been hydrolyzed by this time. The total pentulose content of the solution was only 160 mg, or 81% of the orthophosphate liberated. At present no explanation can be offered for this discrepancy. Chromatography by ascending development in 90% phenol showed that the solution contained only ribulose (R_f 0.66) and xylulose (R_f 0.55), no other sugar being detected by the spray reagents used (18, 21, 22).

The solution was demineralized by passage through a mixed column of Dowex 50 H^+ and IR 45 OH^- resins, the eluate made 0.01 *M* with $Na_4B_2O_7$, and chromatographed on a 20×2.8 (diam.) cm Dowex 1×10 borate column under the conditions described by Dickens and Williamson (24). The fractions containing XU were pooled (1500 ml). Analyses showed that this fraction contained 58 mg of XU, the ratio of optical density 540/670 mμ in the orcinol reaction being 0.46, which agrees well with literature values (9, 18, 26). The xylulose solution was freed from cations, the boric acid removed, the solution decolorized with charcoal and evaporated to a syrup (24, 25). The xylulose was extracted with *n*-propanol to free it from insoluble material which could not be removed by mixed-bed ion exchange resin treatment. The specific rotation of the syrup, based on the xylulose content as determined by the cysteine–carbazole reaction was $[\alpha]_D^{23}$ −21.2° in water (*c*, 1.82), as compared with $[\alpha]_D^{18}$ −33.2° (5) and $[\alpha]_D^{25}$ −36.8° reported for pure D-xylulose (10). Ashwell and Hickman (12) reported a similar discrepancy with xylulose purified from Dowex 1 borate columns. Chromatography with 90% phenol revealed a single zone (R_f 0.67) characteristic of XU. The ratio of color development in 15′/120′ in the cysteine–carbazole reaction was 0.56, which is characteristic of xylulose. This evidence would indicate that the product was XU.

The fractions containing the RU were pooled to yield 1100 ml of solution which, as determined by the cysteine–carbazole reaction contained 38 mg of ribulose. In the cysteine–carbazole reaction color development was complete within 15 minutes, and the absorbancy ratio 540/670 mμ in the orcinol reaction was 0.86. Chromatography of the concentrated solution showed one component (R_f 0.56) which gave reactions typical of RU. These values are similar to those reported for RU by other investigators (9, 18, 26, 27).

The results of this experiment show a ratio of RU:XU of 1:1.5.

Inhibition of PRI by Phosphoribonic Acid

The sensitivity of spinach PRI to phosphoribonic acid was first observed by Axelrod and Jang (28), who noted 50% inhibition by a 1×10^{-4} concentration. In the present work purified PRI was also inhibited by phosphoribonic acid, the inhibition being about 45% by a 5×10^{-3} *M*, and 28% by 5×10^{-4} *M* concentration.

Equilibrium with Purified EPIM

A number of tubes containing 0.05 ml of purified EPIM (0.25 μg protein =

0.0055 units) were incubated with 0.02 ml (0.2 μmole) of NaRU5P at 37° C, four tubes being withdrawn at intervals to determine the XU5P concentration

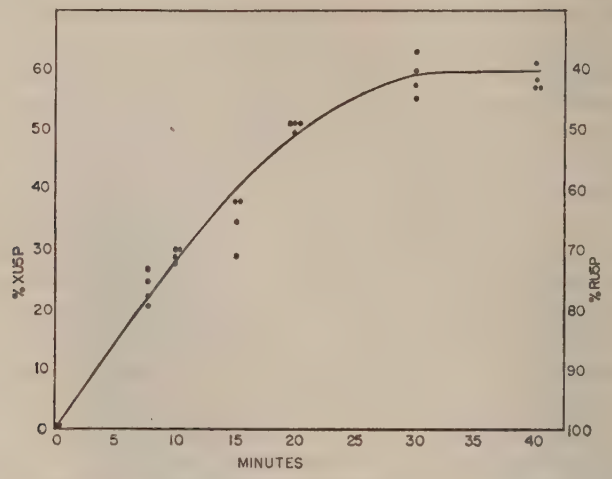

FIG. 4. Equilibrium with purified EPIM; XU5P formation determined by ratio of rate of color development after 15 and 120 minutes in the cysteine–carbazole reaction (see text).

by the cysteine–carbazole method. The results (Fig. 4) show that equilibrium is attained when the ratio of XU5P:RU5P is approximately 1.5:1.0.

Discussion

The equilibrium in the PRI system has been found by most investigators to be from 70–80% in favor of R5P formation and 20–30% in favor of RU5P formation (2, 25, 28, 29, 30, 31). On the other hand, the equilibrium with PRI prepared from erythrocytes was 41.5% RU5P:58.5% R5P, and from rabbit muscle 36–47% RU5P:53–64% R5P (24). In the present work the equilibrium with a fairly highly purified PRI from fish muscle has been found in different experiments to be 40–45% RU5P:55–60% R5P.

There has usually been quite close agreement in the results different investigators have obtained in studying equilibria with EPIM preparations from different sources, namely 40–45% RU5P: 55–60% XU5P (10, 24, 27, 32). On the other hand other investigators have reported ratios of 24–26% RU5P: 74–76% XU5P for EPIM preparations from spinach and muscle (31). In the present work with a fairly highly purified fish muscle EPIM the ratio was about 40% RU5P:60% XU5P. The same ratio has been reported for L-ribulose 5′-phosphate 4′-epimerase (33).

The equilibria in systems containing both PRI and EPIM have been reported as 35% R5P:28% RU5P:37% XU5P (12) and 41% R5P:14% RU5P:45% XU5P (31). The present work has shown that the equilibria obtained with a crude fish muscle preparation containing both PRI and EPIM, and with a

mixture of the fairly highly purified enzymes are similar; namely, 43.5–46% R5P:25.0–26.5 RU5P and 29–30.5% XU5P respectively. These results indicate somewhat higher proportions of both R5P and RU5P and smaller proportions of XU5P than those previously recorded for this system. However, the ratio of XU5P to R5P found in the present work with both mixed purified enzymes and the crude enzyme mixture (pentulose phosphates 53.5–55.7%: R5P 40.5%–44.3%) is very similar to the average values reported for mixed rabbit muscle PRI and EPIM, namely 58.5% mixed pentulose phosphates: 41.5% R5P (24).

The possible reasons for the apparent discrepancies in the ratios of reactants in the above systems have been discussed (31, 32) but so far there has been no really satisfactory explanation for them.

Acknowledgment

I wish to thank Mr. A. P. Ronald for making the specific rotation determinations.

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ANTIBIOTICS AS A PRESERVATIVE MEASURE*

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The methods commonly employed to preserve foods include heating, chilling, freezing, desiccation, exposure to penetrating and other radiations and addition of chemical agents. Due to the serious misuse of chemicals in foods which often occurred before federal government regulations were enforced, some laymen still look askance at foods which contain such additives. This attitude is basically unsound, because all our foods are mixtures of chemical compounds, and most of us continually ingest with impunity small amounts of compounds which could be quite toxic if taken in large concentrations. Thus common salt, acetic acid (vinegar), sodium fluoride (used in water supplies) and sodium nitrite (used in most cured meats) may all cause fatal poisoning if consumed in excess. Food additives include substances which retard spoilage by microorganisms, development of rancidity in fats, or improve flavour or colour. Before such substances may be used in or on foods they must be properly approved by pure food and drugs authorities. It is probably safer, and usually much more pleasant, to consume a food which has been preserved, flavoured or coloured by approved harmless chemical substances, than to consume untreated foods.

There is no hard and fast dividing line between antibiotics and other chemicals. On the other hand, the term antibiotic has been loosely associated with development of hypersensitive reactions and shock in patients and with development of antibiotic-resistant microorganisms so that individuals can

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no longer benefit from treatment. The fact that liberal administration of one antibiotic may be frequently associated with shock reactions in patients is no cause to condemn unreservedly the use of all antibiotics in preservation of foods. There are just as many good reasons why we should use antibiotics in food preservation as we should use them in therapeutic medicine. However, both types of usage are indeed subject to the same general qualification, namely, that improper and uncontrolled use may result in loss of effectiveness.

Early exploratory attempts to preserve flesh foods (Tarr, 1944, 1948; Tarr and Deas, 1948; Hounie, 1950) and milk (Curran and Evans, 1946; Foley and Byrne, 1950) with antibiotics were not very successful, and it was not until 1950 that tests with the "wide spectrum" antibiotics Aureomycin (CTC) and Terramycin (OTC) showed that they were remarkably effective in quite low concentrations in preserving chilled fish (Tarr et al., 1950). The addition of Rimocidin, an antifungal antibiotic, was found to inhibit yeast development which tended to occur on prolonged storage of CTC-treated fish and meat (Tarr et al., 1952). Experiments carried out by Goldberg et al., (1953) with red meats confirmed and extended these observations. It was at about this time that the first promising attempt to reduce the rather severe heating conditions normally required to sterilize canned vegetables by use of the antibiotic subtilin was made (Anderson and Mischener, 1950). More recent experiments along these lines will be referred to later.

For several years practically all work on preservation of flesh foods with antibiotics was carried out in two laboratories, namely the Vancouver Technological Station of the Fisheries Research Board of Canada and the Department of Agricultural Biochemistry, Ohio State University. It is interesting to note that in both laboratories CTC proved the most effective antibiotic studied, followed by OTC and then by chloromycetin. From about 1954 up to the present time increasing interest has been taken in preservation of foods by use of antibiotics, and some of the results of this work will be discussed.

Fish

It was soon realized that, if optimum effectiveness was to be realized in retarding bacterial spoilage of fish with antibiotics, it would be necessary to apply these as soon as possible after capture. It is for this reason that much work has been carried out in which CTC has been incorporated in ice used for icing fish, or in chilled sea water in which they have been transported. Germicides are quite easily incorporated in flake type ice, but it is much more difficult to distribute them uniformly throughout large blocks of ice such as are normally used in the fishing industry (Tarr, 1948). The problem of distributing CTC in blocks of ice has been overcome fairly satisfactorily by addition of certain hydrocolloids to the water used in their preparation, and there have been several different publications and two patents concerning the various substances used (Upham et al., 1954, 1956; Gillespie et al., 1954, 1956; Boyd et al., 1955; Tarr and Gillespie, 1958). The most useful substances have been carragenins and carboxymethylcellulose, but certain alginates are also satisfactory (Moyer, unpub.). The early tests concerning this distribution were made using "soft water", and it was not until problems arose regarding the apparent loss of antibiotic activity in CTC block ice (Shewan, cited by Reay, 1956) that it was realized that the use of "hard" waters presents a special problem. Thus, in waters which are rich in calcium salts relatively water-insoluble complexes form between CTC, the hydrocolloid and calcium ions when the water is frozen. On thawing, curdy precipitates result which, though they contain active antibiotic activity, remove much of the readily available soluble CTC from solution (Moyer et al., 1958). Available chlorine in chlorinated water supplies also inactivates CTC rapidly (Tawara and Sasano, 1957). Demineralization of hard and heavily chlorinated waters by means of ion exchange resin treatments is undoubtedly the most logical means of rendering them satisfactory for use with CTC.

During the past five years very extensive work on preservation of such diverse marine forms as shellfish, fish, and marine mammals has been undertaken in several countries. In British Columbia a number of quite large-scale industrial trials indicated that the keeping quality of salmon (Gillespie et al., 1954, 1955) and "ground fish" (soles, grey cod, Sebastodes sp.) (Boyd et al., 1957a) may be markedly improved by use of flake ice or crushed block ice containing from 1 to 2.5 parts per million (ppm) of CTC. This antibiotic was used successfully to retard bacterial spoilage of salmon (Steiner and Tarr, 1955), halibut (Southcott et al., 1958b) and lingcod (Baker et al., 1958) which were stored in refrigerated sea water. In this medium the actual bacteriostatic effect of CTC was not so marked as it was when ice containing CTC was used, but the antibiotic strongly delayed development of unpleasantodours. Recently, Castell and Greenough have demonstrated the usefulness of CTC and OTC in preservation of eviscerated Atlantic fish (1958a) and fillets (1958b).

In Japan Tomiyama and his collaborators have carried out extensive research concerning preservation of both round and eviscerated fish with CTC (Tomiyama et al., 1955a and b; 1956 a, b, c and d). Their experiments established that such different methods as icing fish with ice containing 5 ppm of CTC, dipping them in water or weak salt solutions containing 10 or 20 ppm prior to storing them at 18° or 20°C or in ordinary ice, or storing them in sea water containing 10 ppm of the antibiotic all caused very significant improvement in their keeping quality.

In the United States Farber and his collaborators have studied the effect of antibiotics on the keeping quality of Pacific fish (Farber, 1954; Farber and Lerke, 1957; Lerke and Farber, 1957; Yakoubovsky-Lerke and Farber, 1956). They have confirmed the fact that CTC is the most effective antibiotic for fish preservation, and have carried out successful commercial studies with this antibiotic. The keeping quality of several commercial species of eviscerated Atlantic fish was considerably

improved by immersing them in solutions containing 10 to 100 ppm of CTC prior to icing them with ordinary ice, or by icing them directly with ice containing 5 ppm of the antibiotic (Firman et al., 1956a; Kline et al., 1957). A simple dip in a solution containing 10 ppm of CTC greatly reduced the rate of bacterial spoilage of fillets of several Atlantic coast fish (Kline, 1958). Stern and his collaborators have indicated that OTC and CTC retard spoilage of eviscerated soles and of salmon (1957a, 1957b) and may be of great value in preserving salmon for canning (Stern et al., 1958). Pasternack et al. (1956) reported favourable results with OTC as a fish preservative.

Very exhaustive studies of the possible value of antibiotics in British fisheries have been made by Shewan and his collaborators (Shewan, 1956; 1957; Shewan and Liston, 1958), who have emphasized the fact that it is only after about ten days storage in ice that marked organoleptic quality improvement is observed in CTC-treated fish. In their experiments, organoleptic tests and several subjective tests were used to assess bacterial spoilage. Shewan and Stewart (1958), in collaboration with investigators at the Cambridge Low Temperature Research Station, carried out a thorough investigation concerning the influence of cathode rays, cobalt 60 gamma irradiation, CTC-treatment and a combination of these on keeping quality of both "fatty" and "lean" fish species. It is interesting to note that at both Stations taste panels gave all irradiated samples (0.25×10^6 to 3.5×10^6 rads.) lower odour and flavour scores than non-irradiated samples, though those given low doses of radiation were considered "acceptable". The results of this experiment cannot be explained briefly, but they did indicate that the CTC treatment used was, in general, more effective than irradiation in retarding development of the total complex viable bacterial flora at 0°C, and that a combination of 0.25×10^6 rads. plus CTC was even more effective than the antibiotic treatment alone.

Reports from Russia (Ravich, 1956; Anon., 1958a) and Greece (Kelaiditis, 1956) have indicated that CTC or other tetracycline antibiotics have proved effective for fish preservation in those countries. In India Valenkar and Kamasastri (1958) found that ice containing 5 ppm of CTC exerted a favourable effect on the keeping quality of several species of fish as assessed by three subjective criteria. In that country recent interesting work has established that, with eviscerated fish stored under tropical conditions (30°C), immersion in solutions containing 25 to 100 ppm of CTC does not improve keeping quality significantly. On the other hand, the keeping quality of fillets at this temperature was about doubled by immersing them for one hour in a solution containing 5 ppm of CTC (Visweswariah et al., 1959). A recent comprehensive investigation in South Africa by Georgala (1959) has established the potential value of CTC-containing ice in extending the storage life of eviscerated hake, and of smoked fillets of these fish when these are treated under specified conditions. Ducroz (1957) has reported favourably on use of 5 ppm of CTC in ice to preserve fish in the French Cameroons under tropical conditions. Kreuzer (1957) obtained favourable preservative effects with fish iced with CTC-containing ice in experiments carried out in Germany. The growth of red halophilic bacteria on salt fish is not inhibited by CTC (Venkataraman and Screenivasan, 1956). It has been reported that tetracycline antibiotics are used for fish and other flesh food preservation in several other countries (Anon., 1957; Shewan, 1957).

Certain investigators have not experienced such pronounced favourable effects with CTC as a fish preservative as have the above workers (Albertsen, 1956; Bystedt and Liljemark, 1956; Hjorth-Hansen, 1956). The reasons for this are presently not clear, but possible explanations are, (1) inability to detect an important improvement in quality of CTC-iced fish during a comparatively brief storage in ice before detectable bacterial spoilage occurred in control fish; (2) lability and destruction

of CTC in the ice used due to such factors as presence of free chlorine or a high pH value of hard water used in preparing the ice, or (3) poor distribution of CTC in the ice used.

Preservation of crabmeat, shrimp and oysters with tetracycline antibiotics has been studied in several laboratories (Tarr et al., 1952; Farber, 1954; Boyd and Tarr, 1956; Fieger et al., 1956; Abbey et al., 1957; Benarde and Littleford, 1957; Farber and Lerke, 1957; Camber, 1955, 1956). The results of these investigations have, in common, shown that considerably higher concentrations of tetracycline antibiotics are required to preserve these shellfish successfully than is the case with fish. Though undoubtedly bacterial spoilage is strongly retarded by the antibiotic treatment, delicate flavoured products, particularly shrimp, lose flavour so rapidly that prolonged chilled storage should be avoided where possible. Large-scale trials have shown that tetracycline antibiotics will preserve whale carcasses successfully when the visceral cavity of these warm-blooded mammals is infused with 50 to 100 grams of the antibiotic soon after capture (Crean et al., 1956; Duncan et al., 1957a and b; Anon., 1958b). Unpublished work (Maxwell, private communication) has shown that sea lion meat may be preserved transiently by use of ice containing about 5 ppm of CTC.

A number of investigators have compared the effectiveness of tetracycline antibiotics for fish preservation. Where careful comparisons have been made at temperatures near 0°C, it has been found that CTC is always more effective than OTC or tetracycline (Tarr et al., 1952; Farber, 1954; Southcott et al., 1958a, Takatsuka et al., 1958; Blackwood et al., 1959). Several investigators have reported that there is no difference in the effectiveness of CTC and OTC for this purpose (Stern et al., 1958; Shewan and Stewart, 1958), or only a slight difference in favour of CTC (Castell and Greenough, 1958b). However, certain of these investigators have not given any detailed description of the experimental methods or conditions used in obtaining the comparative information.

Antibiotics have been used, at least experimentally, for preserving fish products other than those intended for use as human food. Thus, Firman et al. (1956) reported favourable results with application of CTC in preventing spoilage of whole menhaden prior to reducing them for fish meal, and others (Idler et al., 1955; Thomson et al., 1956) have shown that low concentrations of penicillin or CTC will preserve stickwater prior to its use in preparation of condensed fish solubles.

The problems of residues of antibiotics in raw fish and shellfish treated with CTC or OTC by a number of different methods, and of their destruction by heat as in cooking, have been the subject of fairly extensive study (Bissett and Tarr, 1952; Steiner and Tarr, 1956; Boyd and Tarr, 1956; Firmen et al., 1956; Shewan, 1956; Boyd et al., 1957; Abbey et al., 1957; Benarde, 1957; Benarde and Littleford, 1957; Boyd et al., 1957; Boyd et al., 1957b; Tomiyama et al., 1957a and b; 1958a and b; Higashi et al., 1958; Southcott et al., 1958b; Castell and Greenough, 1958c). With iced fish there is a strong tendency for CTC to concentrate in the skin, and to penetrate the flesh slowly. With fish iced with ice containing 5 ppm of CTC the residues in the fish flesh are very low, and would usually range from 0 - 0.2 ppm. Flesh of fish stored in refrigerated sea water containing similar CTC concentrations has higher levels of antibiotic. Most cooking procedures destroy all, or all but traces, of active antibiotic so that fish flesh after cooking would usually contain either no demonstrable CTC or < 0.1 ppm of the antibiotic. Naturally, residues would be higher with types of cured fish or shellfish which might be consumed uncooked. There is no indication that trace amounts of tetracycline antibiotics which might occur in treated fish could in any way impair human health. This aspect of antibiotic treatment of foods will be discussed later. If any real danger exists in the procedure of treating fish with tetracycline antibiotics to improve quality it is because unethical processors might attempt to use them to cover up poor procedures, or because use under unsanitary conditions might permit development of

a predominant flora of the few already antibiotic-resistant bacteria which are normally present on fish and thus invalidate the whole procedure.

Other interesting observations concerning fish preservation with tetracycline antibiotics include the findings that they do not retard trimethylamine nor hydrogen sulphide formation (Castell and Greenough, 1957), and that 70% of the bacteria isolated from certain Indian fish were sensitive to 5 ppm of CTC (Valenkar, 1958).

Poultry

The early success obtained with application of CTC in controlling bacterial spoilage of fish stimulated research into its application to other flesh foods. Ziegler and Stadelman (1955) and Kohler et al. (1955) reported, almost simultaneously, that chilling eviscerated chickens in water containing ice plus 10 to 40 ppm of CTC increased their keeping quality 5 to 7 days longer than that of non-antibiotic treated controls. The last-named investigators found that CTC was more effective than TC or OTC for poultry preservation. Later Broquist et al. (1956) reported the results of experiments carried out in commercial plants in which eviscerated chickens were immersed for 2 hours in ice-water containing 10 to 15 ppm of CTC. Even after 3 weeks storage at about 3°C treated birds were still edible. Ayers et al. (1956) tested a number of antibiotics for their effectiveness in preserving dressed chicken. In their experiments the order of effectiveness in controlling spoilage when used in 3 ppm per million concentration was CTC < TC < OTC, but at higher concentrations this difference was either less outstanding or not demonstrable. Yeasts tended to develop slowly in CTC-treated carcasses, and these could be controlled to some extent by use of certain antifungal antibiotics. A thorough study of the effects of certain practical processing conditions on CTC treatment of eviscerated poultry was made by Kohler et al. (1957). Stadelman et al. (1957) reported that CTC and OTC were equally effective in preserving eviscerated

poultry. Somewhat later Wells et al. (1957) in a careful comparative study found that, for storage at 0 to 12°C, treatment in 10 ppm solutions of CTC was significantly more effective than that obtained with either OTC or TC. Much more effective preservation resulted from antibiotic treatment when the birds were stored between 0° and 3°C. Shannon and Stadelman (1957) found that CTC effected a much greater improvement in keeping quality of CTC-treated chickens when the birds were stored at 0°C rather than at 20°C. Windlan et al. (1957) and Kraft et al. (1958) found that bacterial spoilage in eviscerated turkeys could be markedly retarded by CTC treatment.

Vaughn et al. (1957) in their study of the use of tetracycline antibiotics for poultry preservation under plant conditions found that the storage life of the treated birds was not extended to such a marked extent over that of untreated controls as had been noted by previous investigators. Marked reduction of bacterial growth but no organoleptic improvement with CTC-treated poultry stored for 11 days at about 3°C was recorded by Carlin et al. (1957). The possible danger attending development of pathogenic fungi such as Candida parapsilosis in CTC-treated poultry has been stressed (Njoku-Obi et al., 1957). It is possible that addition of an antifungal substance such as sorbic acid, or an antifungal antibiotic, could prevent such an occurrence. The principal CTC degradation product in cooked CTC-treated poultry is said to be isochlortetracycline (Shirk et al., 1957). In conclusion it is interesting to observe that it has recently been found that cultures of the spoilage bacteria normally associated with poultry usually develop resistance to CTC only after many months of continual transfer in CTC-containing medium (Vaughn et al., 1959). A number of studies concerning the removal of CTC from treated chickens have been made, and it has been shown that the normal cooking procedures used assure total inactivation of the antibiotics (cf. Kohler et al., 1955, 1957).

Meat

The first thorough study of the value of antibiotics in preserving meat was made by Deatherage and his associates (Goldberg et al., 1952; Weiser et al.,1953a and b). Of six antibiotics examined only CTC, OTC and chloromycetin preserved beef effectively, and, of 93 bacteria isolated from meat, 81 were sensitive to CTC, 77 to OTC and 74 to chloromycetin. Infusion of beef rounds via the femoral artery with a solution containing 55 ppm of CTC, or infusion of whole carcasses via one of the carotid arteries, caused a very significant improvement in keeping quality. Intraperitoneal injection of living animals with OTC prior to slaughter has been suggested as a convenient method of preserving carcasses of subsequently slaughtered animals (Wrenshall, 1957). However, Deatherage (1956) found that this method is inclined to be complicated by precipitation of the antibiotic in the peritoneal cavities of treated animals, and by its concentration in the liver and kidneys. Jay et al., (1957a) found that development of a CTC-resistant flora in treated meat was due to a selective increase in the CTC-resistant bacteria already present, and not to a genetic change in sensitive organisms. They have shown that spoilage organisms are very frequently sensitive to comparatively low concentrations of CTC in meat, while they tolerate much higher concentrates in laboratory media. They suggest that this is because in meat CTC forms insoluble chelates with certain of the multivalent cations and anions which are necessary for bacterial growth (Jay et al., 1957b, 1957c). The value of OTC in preserving hams has been assessed by Ordal and Brown (1957), who found that the effectiveness of this antibiotic was improved by moderate concentrates of sodium chloride. Niven and Chesboro (1957) and Cain et al.(1958) have shown that penetrating radiations together with OTC give better preservation of meat than do tetracycline antibiotics alone.

Miscellaneous

Katznelson (1950) found that antibiotics cannot prevent development of American foulbrood of bees. Smith and Hardenburg (1954) and Smith (1955) showed that bacterial spoilage of cole slaw and of spinach can be retarded by rather high concentration of streptomycin (500-1000 ppm). Koch and Carroll (1957) and Carroll et al. (1957) found that OTC was more effective than any of several antibiotics studied in controlling bacterial decay of spinach and other vegetables. Other investigators found that OTC, CTC and streptomycin were all effective for this purpose when spinach was immersed for 1 minute in 25 ppm solutions of these antibiotics (Becker et al., 1958). Geron (1954) has reported that spoilage of grapes is retarded by penicillin. It has been reported that trace amounts of polymixin and penicillin will hinder development of undesirable gram negative and positive bacteria, respectively, in beer fermentations (Strandskov and Brockelman, 1953). Recently, Ayers and Denison (1958) have shown that certain antifungal antibiotics, particularly myprozine and Rimocidin, strongly retard growth of yeasts and moulds in certain fruits. These two antibiotics were also the most effective of several investigated in retarding growth of isolated cultures of fungi which are normally associated with food spoilage (Klis et al., 1959).

One of the early and interesting practical developments is that of the use of the polypeptide antibiotic nisin to prevent the gassy butyric anaerobic fermentations which occur in cheese and processed cheese (Berridge, 1953). In addition to the use of nisin in cheese, many other possible applications have been suggested (Hawley, 1957). One of the most promising of these appears to be a use similar to that of subtilin, namely reducing thermal resistance of spores of anaerobic clostridia and thus to reduce the time and temperatures normally required in canning procedures (O'Brien et al., 1956). However, the whole question of the suitability of antibiotics for this purpose has not apparently as yet been settled

satisfactorily. Hawley (1958) has discussed some of the factors governing the use of nisin as a food additive.

Public Health Aspects and Practical Applications

Several articles have reviewed different aspects of the applications of antibiotics to foods, and certain of these have suggested some problems which might arise from such applications (Campbell and O'Brien, 1955; Barnes, 1956; Ingram et al., 1956; Partmann, 1957; Tarr, 1956a and b; 1957 a and b).

It seems that the chief concerns regarding the use of antibiotics in foods relate to the possibility that their use might; (1) be accompanied by sensitization of individuals with consequent danger of anaphylactoid shock; (2) be followed by an increase in antibiotic resistant organisms in consumers and consequently by a possible lack of response of individuals to antibiotic therapy; (3) result in a cumulative toxicity, and (4) alter the natural microbiological flora of the food or of the consumer unfavourably so that either pathogenic organisms might develop in the food, or the microbial flora of consumers be altered leading to unpleasant reactions. It hasbeen pointed out that some of these questions can be answered better by experience than by experiment (Ingram et al., 1956). However, much useful information relative to these questions has already accumulated. Thus, so far as can be ascertained the fairly extensive use of CTC in foods such as poultry and fish has not as yet been accompanied by hypersensitive reactions in consumers. This is not entirely unexpected for, although significant penicillin residues may be present in as many as 5% of samples of market milk, it has been stated that no case of a hypersensitive reaction has yet been positively traced to consumption of such milk in the United States (Welch, 1957). Penicillin is the one antibiotic to which many individuals become readily sensitized.

The use of antibiotics in flesh foods will usually result in no active residues in the cooked materials, or if there are active residues they will be to

all intents and purposes negligible. It has now been common commercial practice for almost a decade to incorporate about 20 ppm of tetracycline or certain other antibiotics in the rations of many different types of farm animals. This practice, so far as can be determined, has not lead to any of the undesirable effects which were originally predicted for such use. On the contrary, their use is in very many instances accompanied by favourable effects on growth and health of the animals (Jukes, 1955), Moreover, rather prolonged administration of about 50 mg per day of tetracycline antibiotics to children has also not been attended by demonstrable unfavourable reactions (Jukes, 1956; Loughlin et al., 1958a and b). The excellent review by Hines (1956) indicates that consumption of comparatively large amounts of tetracycline antibiotics by infants, adults and geriatric patients has never been accompanied by cumulative toxic reactions. At therapeutic dose levels, which are thousands of times larger than those which might occur in treated foods, there may be transient unfavourable reactions, or the intestinal flora may be adversely affected (Welch et al., 1958). So far, treatment of flesh foods with antibiotics does not seem to have caused development of pathogenic or other undesirable microorganisms. It has been demonstrated that growth of Clostridium botulinum (type E) and an enterotoxigenic strain of Staphylococcus aureus was retarded strongly in fish flesh treated with CTC (Bluhm and Tarr, 1956; Boyd et al., 1957a). The danger of development of pathogenic fungi in treated poultry which has already been referred to has so far apparently not materialized in practice, and this also appears to be true of salmonellosis (Ingram et al., 1956). It is hoped that further work will be carried out along these lines. The real dangers of use of antibiotics in foods appear to be; (1) application with the idea of unnecessarily prolonging "shelf life" so that the consumer never receives an article of improved quality, and (2) application under poorly supervised and unsanitary conditions so that antibiotic-resistant bacteria develop, consequently rendering the process useless for its intended purpose; treatment of already partially spoiled foods, a procedure

which has been shown, in several laboratories, to be either ineffective or nearly so.

In conclusion, it is of interest to mention the officially permitted applications of antibiotics in foods on the North American continent. On November 30, 1955, the United States Federal Register established a tolerance for CTC levels on chicken (Anon., 1956) and on April 21, 1959 (Anon., 1959) tolerances for use of CTC on certain fish products as follows:

(A) 7 parts per million in or on uncooked poultry. This tolerance level shall not be exceeded in any part of the poultry.

(B) 5 parts per million in or on fish vertebrate, scallops, shucked, shrimp unpeeled, from application for retardation of spoilage to whole, headed, or gutted fish vertebrate, scallop shucked, shrimp unpeeled, each in fresh, uncooked, unfrozen form.

In Canada, the Regulations under the Pure Food and Drugs Act were amended in 1956 and again in 1959, and now read as follows:

"B.21.07. No person shall sell fish that has in or upon it chlortetracycline or oxytetracycline unless

(a) its presence or the presence of both, if both are used, is declared by name on the main panel of the label, and

(b) the total amount, singly or in combination, thereof does not exceed 5 parts per million."

CTC and OTC are also permitted for poultry preservation in concentrations which must not exceed 7 ppm of the raw product. Japan permits use of CTC for certain fish intended for use in preparation of kamaboko, and a number of smaller countries also permit use of tetracycline antibiotics for preservation of red meats, fish and poultry (Anon., 1957).

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AGE DETERMINATION IN MOOSE FROM SECTIONED INCISOR TEETH

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The purpose of this paper is to present some of the results of a preliminary study in which we aged moose (*Alces alces*) by use of growth layers in the cement of incisor teeth. The techniques used in the study will also be discussed.

We wish to acknowledge the assistance of Arthur W. Mansfield of the Fisheries Research Board of Canada, who criticized the manuscript and aided in the preparation of the figures.

Laws (1952, 1953) developed a method of age determination in seals by preparing thin sections of the canine teeth and examining the regular sequence of growth layers in the dentine. This method has now been applied to a number of species of pinnipeds (e.g. Fisher, 1954; McLaren, 1958) and also odontocete whales (e.g. Nishiwaki, Hibiya, and Ohsumi, 1958). Some species, such as the fur seal (*Callorhinus ursinus*), show external ridges over the root of the canine tooth. Scheffer (1950), using marked fur seals of known age, showed that these ridges were laid down annually. The ridges correspond with growth layers in the dentine but are not so useful for aging, since they become obscured within a few years by deposition of overlying cement. The cement of marine mammal teeth also shows growth layers, but more commonly than not, deposition is very slight. Where the cement is thick, layers can be counted and then provide a check on counts of dentine layers.

Not all pinnipeds and odontocetes show regular growth layers in the teeth. It seems likely that the alternation of different types of deposition is related primarily to the

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feeding cycle, but endocrine rhythms possibly play a part. Due to lack of known-age animals, the annual sequence of growth layers has generally been determined by examination of specimens taken at different times of the year.

Laws (1953) briefly noted the occurrence of external growth ridges on the teeth of a variety of land mammals and of fossil reptiles. For this reason, we took the opportunity to explore the cement layers as age indicators in moose teeth that Pimlott had collected in Newfoundland. Sergeant's preliminary examination of a few incisors showed that growth layers could be seen in the cement, with less marked periodic growth in the dentine. A larger sample was accordingly prepared and examined. The material came from 64 moose taken in Newfoundland between 1950 and 1956, the majority in the December hunting season, but some at other times of the year. Pimlott had previously aged all these animals, using the method developed by Passmore, Peterson, and Cringan (1955), which is based largely on the extent of wear of the mandibular teeth. Yearlings can be accurately aged by this method, not only because the teeth are very little worn, but also because some teeth are incompletely erupted. Older animals are aged from mandibular tooth wear alone. We might expect this method to be reliable for the younger age classes but less reliable with increasing age, owing to variations in rate of wear.

TECHNIQUES

Since the first incisor is the largest of the four incisiform teeth and erupts first, it was chosen for age determination. In large-scale removal of incisors, the anterior portion of the mandible was removed and cooked in either a pressure cooker or an autoclave for approximately 45 minutes at 15-lb. pressure. When the autoclave was used, it was found necessary to immerse the mandibles in a water bath to prevent the cement of young teeth from cracking and peeling. Teeth were bisected longitudinally, using either a jeweler's circular saw blade or a band saw. The saw blade (obtained from the Thurston Manufacturing Co., 45 Borden Street, Providence, Rhode Island) was mounted on a grinding head and had a 3-in. diameter, 0.20-in. thickness, and ½-in. central hole with 24 teeth to the inch; but a larger blade might well be used. The band saw was equipped with a 24-tooth, ½-in. metal cutting blade.

We found that the blades lasted much longer if the crown of the tooth, which has an exceedingly hard enamel, was not sectioned. The cut, therefore, started at the root and ran forward to the base of the crown, which was then removed with a transverse cut. To protect the operator's fingers from injury, each tooth may be held in a pair of pincers or jammed in a wooden block while being cut. To prepare sections, the cut half-tooth may be cemented with plastic wood to a wooden block, suitably shaped to receive it. A thick section is then cut on the circular saw as before. This section is ground to translucency on a fine carborundum or aluminum oxide stone. In this study, the rather elaborate grinding machine described by Fisher and Mackenzie (1954) was used, consisting of two vertically mounted grindstones, one fixed and one rotating, driven by a motor and cooled with water. Such a machine is of great value when a large number of tooth sections are to be prepared, although grinding by hand is not too laborious for a few sections.

The polished faces of half-teeth were moistened with alcohol and examined at magnifications of 10× to 40×, using a Zeiss binocular microscope. A calibrated micrometer scale was attached to one eyepiece to measure thickness of the growth layers. Under reflected light, opaque material appears white, while translucent material absorbs light and appears dark. Thin sections were mounted in alcohol and examined under the low power of the compound microscope, with condenser removed to allow light scattering. With transmitted light, opaque material appears dark and translucent material light. To avoid confusion, the terms opaque and translucent are used in our paper to describe the contrasting optical properties of the tooth tissue.

In measuring the thickness of the cement layers, care must be taken that the section lies close to the median longitudinal plane of the tooth, since, with an oblique or tangential section, a greater thickness is obtained. It may also be noted that the thickness of the cement is greater on the posterior, concave face of the incisor than on the anterior, convex face. Our measurements refer to the concave face.

RESULTS

The first permanent lower incisors of Newfoundland moose normally erupt in December or January, when the calf is 7 or 8 months old. Presumably these teeth have begun growth and are beginning to calcify about the time of birth.

Fig. 1 shows whole views of first incisors of a young and an old animal. The root of the incisor of the young animal is tapered, but in the old animal it is thickened and rugose. Fig. 2 shows longitudinal halves of sectioned incisors with the crowns removed. It can be seen that cement deposition takes place over the whole root, thinning out toward the cemento-enamel junction and the base of the root, and is thickest about two-thirds of the way toward the base. In the oldest animals, cement deposition becomes irregular, producing the rugose bosses seen in whole view. Only the localized thickenings of the cement show the full sequence

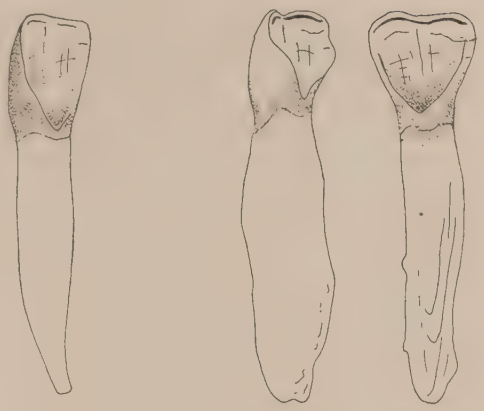


FIG. 1. Drawings of first incisors of a 1-year-old (left) and a 13-year-old moose (right) to show the general form, the cemento-enamel junction separating crown and root, and the increase in thickness of the cement that takes place with advancing age. Magnification 1.1×.

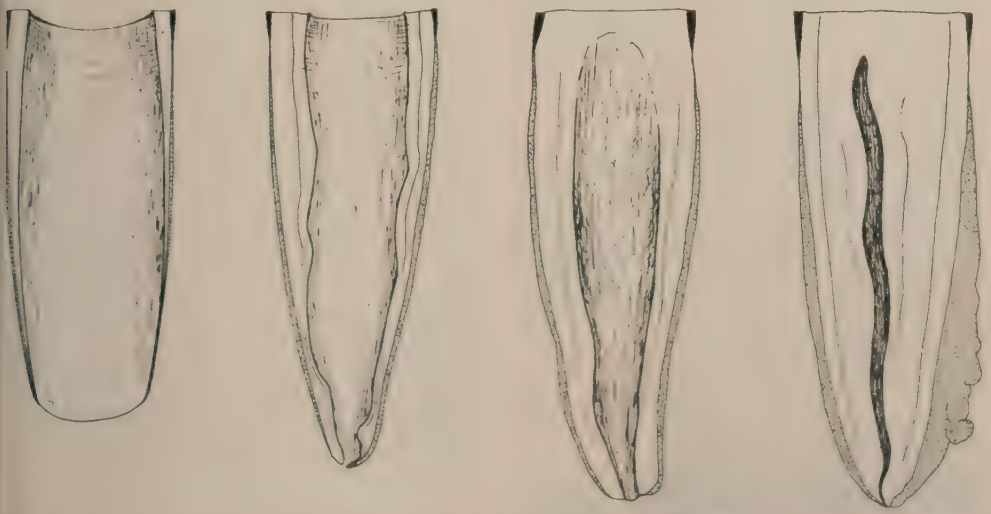


FIG. 2. Drawings of longitudinal halves of sectioned moose incisors, with the crowns removed. From left to right: a calf aged 6 months, a 1-year-old, a 3-year-old, and a 15-year-old animal. The series illustrates the increase in thickness of the dentine and consequent narrowing of the pulp cavity that occurs with age, and the form of the cement layer, which slowly increases in thickness. White—dentine; stippled—cement; black—enamel. Magnification 2.3×.

of growth layers. This explains why longitudinal, rather than transverse, sections were used in studying the sequence of growth layers.

The dentine grows inward throughout life and gradually obliterates the pulp cavity (Fig. 2). However, a small pulp cavity remains in all except some of the oldest animals, indicating that dentine deposition continues almost throughout life. In sectioned teeth, dentinal growth layers were detectable from the alternation of opaque and lighter colored zones. However, the boundaries of dentinal growth layers were indistinct and counts of their number tended to be subjective. For this reason, only cement layers were used as a guide to age in this study. Dentine layers would probably repay further study, using better methods of differentiation such as polarized light and staining with haematoxylin.

Growth layers in the cement (Fig. 3) consist of alternating opaque and translucent zones spaced at fairly regular intervals. The opaque zone is generally the wider of the two. In thin section, it can be seen that the layering is caused by a periodic concentration of cementoblasts. In macerated teeth the empty cell bodies of the cementoblasts are filled with air, and when the section is wetted the mass of air cells refracts the light. The opaque zone is thus the zone of great cementoblast frequency.

The earliest growth of the cement, next to its junction with the dentine, was measured in the first incisor of 3 calves about 6 months old and taken in December, i.e., at about the time of eruption of these teeth. The thickness of cement was about 0.12 mm. Apparently a single opaque zone had been laid down against the border of the dentine, followed by a thin translucent zone, but the sequence is difficult to pick out in these very small, hard-to-section teeth.

The tooth of a 1-year-old taken in late May showed little change in diameter or

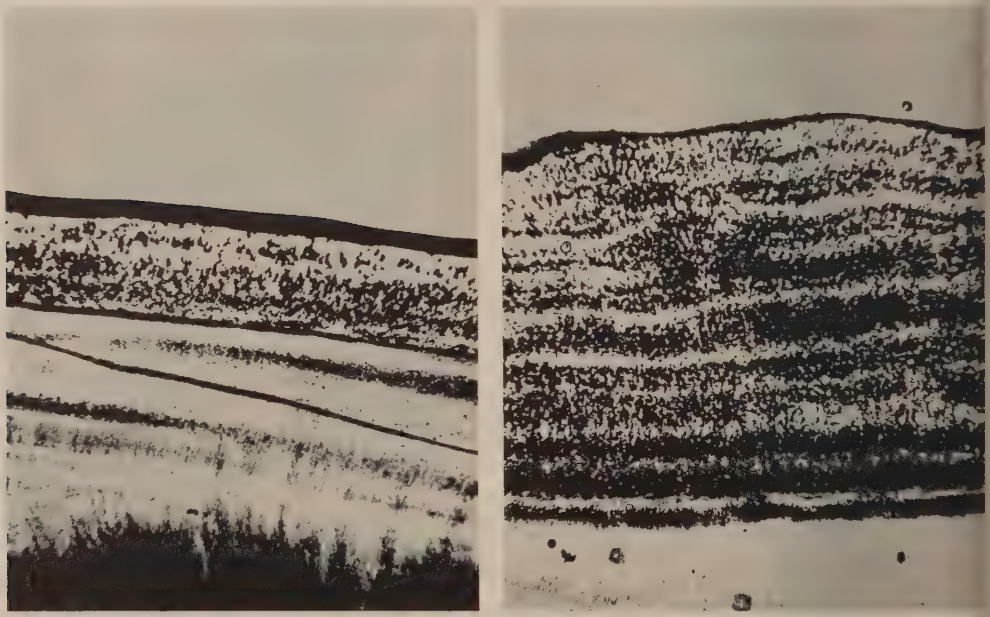
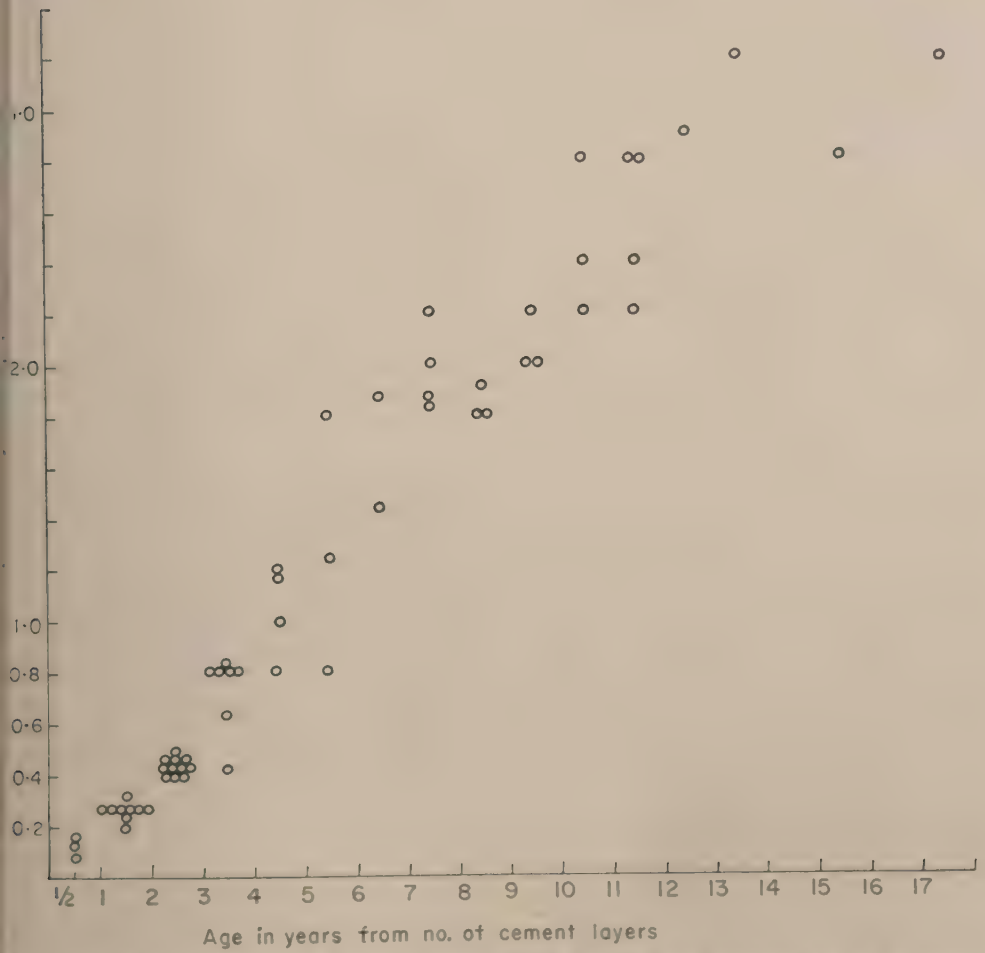


FIG. 3. Thin longitudinal sections of two moose incisors to show the structure and layering of the cement. Low-power photomicrographs without condenser and using a green filter. At left, a class III animal aged at 4 years. The sequence ends with a translucent zone in this December-killed animal. At right, a class VI animal aged at 15±2 years. Older layers gradually decrease in thickness.

ucture of cement. The teeth of 9 yearlings (8 months old) taken in December showed ment with a thickness of 0.20–0.32 mm. o complete growth layers had been med, the sequence beginning with an aque zone and ending with the second nslucent zone. It seems reasonable to ppose that the second opaque and trans- ent zones are formed between the first d second winters, in which case the first nslucent zone must be laid down in the st summer or fall and the first opaque ne probably about the time of birth. The ole of the first layer has a speckled appearance not seen in later layers, which seems to indicate a different type of calcification. Moreover, its diameter (0.12 mm.) is less than that of succeeding layers (0.2 mm.). These two considerations suggest that the first layer is indeed laid down before the incisor erupts in the first winter of life.

The early growth layers from age 1 to about 10 years have a fairly constant thickness, giving a linear curve of increase in thickness with time (Fig. 4). Measurement of the maximal thickness of cement can thus help to check the age of those young animals



g. 4. Graph showing increase in maximal thickness of the cement with increasing age in the moose. he annual deposition is approximately constant from age 1 to about 8, at a rate of 0.2 mm. per annum.

TABLE 1. COMPARISON OF AGES DETERMINED FROM MANDIBULAR TOOTH WEAR AND FROM NUMB OF CEMENT LAYERS IN MOOSE

TOOTH WEAR			CEMENT LAYERS														
			No. Falling in Each Age (by Years)														Known Range of Age Class in Years
Age Class[1]	No. in Sample	Est. Age (Years)	1	2	3	4	5	6	7	8	9	10	11	12	13	15	17	
I	11	1	11															1
II	15	2		12	3													2–3
III	8	3			6	2												3–4
IV	8	4				4	2	1	1									4–7
V	7	5–6						1	3	1	1	1						6–10
VI	9	6–8								1	3	2	2			1		8–15
VII	6	8–10										1	2	1	1		1	10–17

[1] Based on technique described by Passmore, Peterson and Cringan (1955).

in which the exact number of growth layers is in doubt.

The greatest age that we found in our sample of moose teeth was 17 years. At this age, the cement is of a very uneven thickness, with irregular bosses containing amorphous spherical inclusions. These inclusions obscure the growth layers in the bosses, but by looking at the margins of the bosses where the cement is not so thick, one can count the entire sequence of growth layers. Farther away from the bosses, the number of growth layers is incomplete.

DISCUSSION

Our estimates of age for classes I and II (Passmore, *et al., op. cit.*) differed only slightly from estimates based on wear, but divergence increases for older animals (Table 1). In the last 3 tooth-wear classes (V, VI, and VII), the newly determined ages of only 3 out of 22 animals fell within the previously estimated range for the class. We did not sample any animals in classes VIII or IX, which were very rare in the Newfoundland collection. Our results might have been different if we had sampled the Ontario moose which Passmore, *et al.* (*ibid.*) used in their original study. Pimlott found some fundamental differences in the tooth erosion of Newfoundland moose, as compared with Ontario moose. For example, the posterior buccal crest of the second lower molar M_2 in Newfoundland yearlings was consistently higher, narrower, and less eroded than in Ontario yearlings collected at approximately the same time of year. In spite of these differences, the variation i ages determined from tooth wear and fro cement layers is so great that doubt thrown on the ranges of the upper ag classes of Passmore, *et al.* (*ibid.*).

After our data were worked up, the firs incisor of a moose of known age was mad available to us by R. Y. Edwards of th Department of Recreation and Conserva tion, Victoria, British Columbia. This ani mal had been tagged as a calf in May 195 and was shot in October 1957, thus bein 3½ years old at death. In section, th cement showed 3½ growth layers, the se quence beginning with a wide, opaque zon and ending with an opaque zone (Fig. 5) This validates our age scale and agrees wit

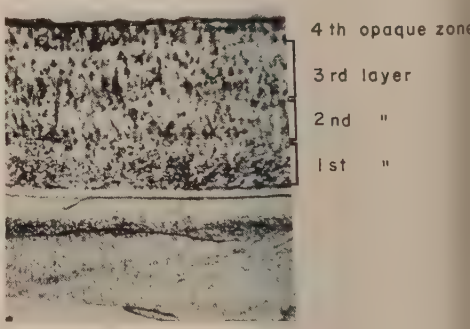


FIG. 5. Thin longitudinal section of the cement o the first incisor of a known-age, 3½-year-old moos from British Columbia. The animal was tagged a a calf in May 1954 and shot in October 1957. Th basal layer of the cement has a fine structure, dif ferent from later layers. The opaque layers ar seen to be delimited by the density of cementoblast The series ends with an opaque layer.

suggested seasonal sequence of deposition (*see* below). However, until more known-age specimens become available to show a further check on the accuracy of the method, we believe it wise to consider the range of accuracy to be plus or minus 1 year for younger animals, and 2 years for older animals. This caution is suggested for two reasons: first, because the first layer varies considerably in appearance and thickness probably depending on the time of eruption of the tooth); and second, because in older animals the later growth layers are too small for accurate counting.

So few animals were taken at other times of the year than December that the seasonal sequence cannot yet be evaluated clearly. The tooth of the known-age animal, however, showed opaque cement in process of deposition in October, apparently nearly complete (Fig. 5). December-killed animals showed early deposition of translucent cement in progress (Fig. 3). Assuming that cement deposition occurs throughout the year, therefore, it seems likely that opaque material is laid down in summer and fall, translucent cement in winter and spring. It is tempting to relate this sequence to seasonal variations in feeding conditions, but more material is needed to confirm the sequence suggested.

Incisors from two Newfoundland caribou (*Rangifer caribou*), sent to us by A. T. Bergerud of the Newfoundland Department of Mines and Resources, showed exactly the same structure as the moose incisors, and provisional ages were assigned from cemental growth layers. However, the much smaller size of the teeth required the use of higher magnification to distinguish the layers, and the cementoblasts were so large a proportion to the layers that they tended to obscure them.

SUMMARY

Incisor teeth from 64 moose taken in Newfoundland were aged, using a method previously developed for marine mammals. The root of the tooth was sectioned and the structure of dentine and cement examined. The cement showed regular alternations of opaque and translucent growth zones. The zones were formed by periodic concentrations of the cell bodies of the cementoblasts. A full annual layer was found to consist of one opaque and one translucent zone. This conclusion was confirmed by examination of an incisor from a known-age ($3\frac{1}{2}$-year) moose, which showed $3\frac{1}{2}$ layers of cement. The seasonal sequence of cement deposition was not elucidated from the limited material, but it seems probable that opaque cement is laid down in summer and fall, translucent cement in winter and spring. Ages determined for younger animals agreed with ages determined independently by the method of mandibular tooth wear, but ages of older animals were consistently higher using the new method. Incisors of caribou showed the same structure as those of the moose, but since the teeth were smaller, the growth layers in the cement were difficult to read.

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BIOCHEMICAL STUDIES ON SOCKEYE SALMON DURING SPAWNING MIGRATION

VII. STEROID HORMONES IN PLASMA[1]

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Abstract

Cortisone, cortisol, and 17-hydroxyprogesterone were isolated from the plasma of prespawning Fraser River sockeye salmon. The female plasma contained double the concentration of cortisone and cortisol found in male plasma, 41 μg and 26 μg/100 ml respectively as compared with 22 μg and 11 μg/100 ml.

Corticosterone is probably present at a very low concentration and neither 11-desoxycorticosterone nor aldosterone was detected in the plasma from approximately 100 fish. Cortisone, cortisol, and total 17-hydroxysteroids were quantitatively determined in plasma samples obtained from two pure races of sockeye captured at various stages of sexual maturity. The combined concentration of cortisone and cortisol which, at the mouth of the river was three times that of the normal human, increased to as high as 17 times this level following spawning. The results are discussed in relation to the death of the fish.

Introduction

Cortisol has been conclusively established to be the principal adrenal cortical hormone in the peripheral plasma of the normal human (1). In general, total 17-hydroxysteroid determinations on human plasma have given higher values than have paper chromatographic methods involving the isolation of cortisol. A recent report describes a mean cortisol concentration in normal male plasma of 7.7 ± 3.5 μg/100 ml when cortisol was determined by paper chromatography as compared to 20.0 ± 8.7 μg/100 ml when total 17-hydroxysteroids were determined (2). Cortisol is also reported to be the principal steroid in the blood of several animals (3) and has been tentatively identified in carp plasma (4). Extracts of the plasma of the Atlantic salmon (*Salmo salar*) have been demonstrated to possess considerable glycogenic activity (5). The same investigators determined the total 17-hydroxycorticosteroid levels by the Nelson-Samuels methods and reported that there was considerably more glycogenic activity than could be accounted for on the basis of the chemical analytical procedure. Attempts to find cortisone in adrenal vein blood (6), where the level of cortisol is extremely high, and in human peripheral blood (7) have been unsuccessful. Occasional blood samples have been reported to contain small amounts of a "cortisone-like" substance but the evidence is inadequate to establish identity (8, 9).

The isolation of 17α-hydroxyprogesterone from the human ovary has been described (10).

The present report is concerned with the isolation and characterization of cortisol, cortisone, and 17-hydroxyprogesterone from sockeye salmon plasma

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and with the quantitative determination of plasma steroids in sockeye at various stages of sexual maturity.

Experimental

Collection of Samples

All sockeye were captured with a dip net. The locations at which the various samples were taken are listed in Tables I and II. The isolation and characterization studies were carried out on the plasma obtained from several hundreds of female salmon captured October 28, 1958, near Lytton, British Columbia, where the fish were about one week from the spawning grounds.

The 1957 Stuart Lake fish were captured at Lillooet, 415 miles from the spawning area and on arrival on the spawning grounds at Forfar Creek, British Columbia (11). All fish were bled by severing the caudal artery and care was taken to exclude slime. Heparin was used as an anticoagulant for the samples taken on the Stuart Lake run while a mixture of ammonium and potassium oxalates was used on the Adams River samples. Samples were centrifuged immediately and the plasma was stored at the temperature of dry ice until analyzed. The temperature of the plasma samples did not exceed 4° C during the thawing process.

Purification of Materials

Solvents were reagent grade. All low-boiling solvents, chloroform, methanol, benzene, etc., were distilled through a suitable fractionating column to assure no residue on evaporation. Chloroform was always distilled immediately before use. Formamide and ethylene and propylene glycol were distilled under high vacuum. The distilled formamide was stored in a desiccator over concentrated sulphuric acid. Specially purified Whatman No. 1 filter paper was used for all paper chromatograms. The paper was extracted in a Soxhlet apparatus for 1 day with 5% ammonium hydroxide; this was followed by a 2-day extraction with 5% aqueous acetic acid, and a 3-day extraction with methanol. The paper was then dried *in vacuo* at a relatively low temperature and stored in Mylar plastic bags to exclude atmospheric contaminants.

Analytical Methods

The dihydroxyacetone side chain of the steroid hormones was determined with the phenylhydrazine reagent (12). The α-ketol determinations were carried out with blue tetrazolium as described by Mader and modified by Morris (8). Steroids with a Δ^4-3-ketone structure were detected on paper chromatograms with ultraviolet light and the location confirmed with the Δ^4-3-ketone specific alkali spray of Bush (1). The blue tetrazolium spray was used to detect steroids with the α-ketol side chain on paper chromatograms (13). The Zimmermann reagent was used to locate 17-keto steroids on paper chromatograms (14) and examination under ultraviolet light before and after alkali spray was used to locate those 17-keto steroids which also contained a Δ^4-3-ketone grouping. All spectra were recorded with a Beckman DK spectrophotometer. Semimicro (1 ml) cells were employed for most determinations.

Quantitative Analysis of Steroids

A fairly specific acid fluorescence method was used for the determination of cortisol and corticosterone (15). Quantitative determinations of cortisol and cortisone were also carried out with the Porter–Silber reagent. Total 17-hydroxysteroids were determined by the method of Silber and Porter. It was observed that a slight yellow color was formed immediately on the addition of the phenylhydrazine reagent to the plasma extracts. This color was not formed by the addition of acid alone nor was it produced by pure reference steroids, therefore a small additional correction not described in the published procedure was applied (12). The authentic steroids referred to are U.S.P. chromatographic reference standards.

Isolation and Characterization of Cortisone, Cortisol, and 17-Hydroxyprogesterone

In a typical isolation 1760 ml of plasma obtained from female sockeye captured at Lytton, B. C., was extracted four times with 2000-ml portions of ethyl acetate. The ethyl acetate was concentrated to 2000 ml in a flash evaporator at approximately 40° C with the receiver immersed in dry ice – acetone. The ethyl acetate (2000 ml) was washed three times with 100-ml portions of 5% aqueous sodium carbonate. The ethyl acetate was then washed with three 100-ml portions of water and the ethyl acetate phase clarified by the addition of small quantities of benzene (7). The residue was then taken to near dryness *in vacuo*. The residue was taken up in 300 ml of 70% aqueous methanol and extracted four times with equal volumes of *n*-hexane. The hexane phase was then washed with 200 ml of 70% methanol which in turn was washed four times with hexane. The methanol phases were combined and diluted with two volumes of water and the mixture was continuously extracted with peroxide-free ether for 24 hours.

Whatman No. 1 filter paper was impregnated with a 30% solution of formamide (16). The residue obtained after removal of the ether was taken up in a small volume of chloroform:methanol 1:1, divided in two portions, and each was applied at the origin on a 12-cm strip of paper. Cortisone, cortisol, and corticosterone were run as reference standards. The chromatograms were developed with chloroform:benzene 1:1 until the solvent had reached the bottom of the paper (17). A strip containing the standards and 1/40 of the plasma strips was examined under ultraviolet light, then sprayed with the alkali fluorescence reagent, and finally with the blue tetrazolium reagent. The plasma strips showed two strongly positive areas for all three tests. One corresponded with cortisol and the other with cortisone (or aldosterone). Visually cortisone appeared to be the major component. The remainder of the plasma paper strips were divided into six sections; one contained the origin and residual fatty material, two contained cortisol, three contained cortisone, four contained the region between cortisone and corticosterone, five corresponded to corticosterone, and the lower limit of corticosterone to the bottom of the paper was number 6.

The materials in sections 1 to 6 were eluted from the paper with three portions of hot methanol. Approximately 20-ml portions were used for the smaller

strips and 40 ml for the larger. The methanolic extracts were transferred to a titerlenmeyer (a 50-ml Erlenmeyer flask with a well 0.8 mm inside diameter and 3 cm deep attached to the bottom), and evaporated to dryness at 59° C under an atmosphere of nitrogen (18). The formamide was then removed under high vacuum at a temperature of about 70°. The contents of the flask were rinsed into the well with chloroform:methanol 1:1 and evaporated again under nitrogen. This procedure was repeated two or three times. The residue in the well was then taken up in several small portions of hot chloroform, the residual paper removed by filtration, and the filtrate was evaporated to dryness.

Fraction 2

Fraction 2 showed an absorption maximum at 239 mμ, and calculated on ϵ 15.8×10^3 and using the molecular weight of cortisol, there was 491 μg of steroid. However, by the more specific acid fluorescence method for cortisol there was only 311 μg or 63.4%. Fraction 2 was then run on paper in the toluene – propylene glycol system for 78 hours (13). Three ultraviolet-absorbing bands were then observed, 2A, 2B, and 2C. The major component was 2C and it had an R_f value identical with that of authentic cortisol. The solvents were removed as described above and substance 2C was shown to be cortisol as described below.

Fraction 3

Fraction 3 showed an absorption maximum at 237 mμ and, based on ϵ 15.8×10^3 and using the molecular weight of cortisone, there was 650 μg of steroid. Fraction 3 was rechromatographed in ethylene glycol – *n*-butylacetate along with authentic cortisone and aldosterone (16). A test strip showed that there was only one ultraviolet-absorbing compound and this corresponded in position to cortisone. This substance was also the only material which exhibited alkali fluorescence or reduced blue tetrazolium. The area corresponding to authentic aldosterone will be referred to as fraction 3A and the area corresponding to authentic cortisone as fraction 3B. Fraction 3A was rerun in toluene – propylene glycol and the aldosterone–cortisone area was bio-assayed for aldosterone activity as described below. Fraction 3B was homogeneous in the toluene – propylene glycol system and in 48 hours travelled exactly the same distance as cortisone.

Fraction 5

Corticosterone, if present, would be in fraction 5. The paper strip exhibited no visible ultraviolet absorption in this area, so to aid in the detection of corticosterone a further 400 ml of plasma was worked up as described above giving a total fraction 5 from 2160 ml of plasma. Fraction 5 was rechromatographed in toluene – propylene glycol and the area corresponding to authentic corticosterone (R_f 0.2) was cut out and eluted from the paper. The sample exhibited no maximum in the region of 240 mμ. The absorption due to the paper was linear and a calculation applying Allen's correction indicated a total of only 1.2 μg of ultraviolet-absorbing material as compared with an appropriate paper

blank (19). If this entire absorption was due to corticosterone it represented a level of only 0.05 μg/100 ml of plasma. The remainder of the paper contained no alkali fluorescent compounds.

Fraction 6

Even after hexane extraction the 70% methanol fractions always contained a certain amount of colored fatty material which ran near the solvent front in the chloroform–benzene system. Therefore fraction 6, which contained all steroids less polar than corticosterone was rerun in benzene: heptane 1:1 – formamide. Three ultraviolet-absorbing compounds were observed, only one of which will be considered in the present report. This material designated fraction 6B ran in a similar position to authentic 11-desoxycorticosterone (R_f 0.45). This material exhibited an absorption maximum at 239 mμ, and corresponded to 204 μg (19 μg/100 ml for 60% recovery) based on the constants for 11-desoxycorticosterone. However, the material was observed to exhibit a sulphuric acid chromogen different from 11-desoxycorticosterone (20). Fraction 6B was rechromatographed in normal hexane – propylene glycol and the ultraviolet-absorbing compound was found to have an R_f value relative to 11-desoxycorticosterone of 0.58, a value identical with that obtained for authentic 17α-hydroxyprogesterone. There was no ultraviolet-absorbing substance corresponding to the 11-desoxycorticosterone.

Identity of Fraction 2C with Cortisol and 3B with Cortisone

Once the chromatographic homogeneity of 2C and 3B had been established, a sample of each was treated for 2 hours at 22° C with concentrated H_2SO_4 (20). Substance 2C exhibited maxima at 237, 282, 395, and 478 mμ, and O.D. ratios of 0.98:1:0.47:0.55, and substance 3B exhibited maxima at 284, 343, and 420 and O.D. ratios of 1:0.38:0.31. These values were identical with those obtained from authentic cortisol and cortisone respectively. Aliquots of 2C and 3B gave quantitative Porter–Silber reactions for the dihydroxyacetone side chain of the magnitude expected from the concentration based on ultraviolet absorption measurements and the chromogens gave the expected wavelength maximum at 410 mμ. Authentic cortisol and 2C were treated with fuming sulphuric acid for 1.5 hours and the spectra of the resulting chromogens were identical, with absorption maxima at 240 mμ and 490 mμ and a shoulder at 270 mμ (21).

Oxidative Removal of the Ketol Side Chains of 2C and 3B

Sodium bismuthate (1 g) was added to ca. 30 μg of 2C, 3B, and authentic cortisol and cortisone, each of which was previously dissolved in 5 ml of 50% aqueous acetic acid. The mixtures were shaken for one-half hour and then filtered through purified Whatman No. 1 paper. The residues and papers were washed thoroughly with water. The aqueous acetic acid filtrates were extracted with chloroform. The chloroform was removed under a stream of nitrogen and the residues were run in heptane:benzene 1:1 – formamide. The reaction products of authentic cortisone and 3B produced a single ultraviolet-absorbing spot with the same R_f value as Δ^4-androstene-3,11,17-trione. Authentic cortisol and 2C both gave a major ultraviolet-absorbing substance correspond-

ing in R_f value to 11-β-hydroxy-Δ^4-androstene-3,17-dione, and both gave also a very minor component corresponding to Δ^4-androstene-3,11,17-trione. The major ultraviolet-absorbing components were eluted from the paper as described above and rerun in benzene:hexane:methanol:water 33:66:80:20 (1). The oxidation products of authentic cortisol and cortisone had R_f values of 0.16 and 0.23 respectively and both compounds absorbed in the ultraviolet and gave positive Zimmermann reactions. The oxidation products of substances 2C and 3B corresponded identically with those from the respective reference standards.

The infrared spectra of substances 2C and 3B confirmed their identity with cortisol and cortisone respectively.

Identity of Substance 6B with 17-Hydroxyprogesterone

A solution of substance 6B in methanol contained 18.3 μg/ml of 17α-hydroxyprogesterone as indicated by the ultraviolet absorption at 240 mμ. The solution of substance 6B and an equivalent solution of authentic 17α-hydroxyprogesterone gave the same quantitative Porter–Silber reactions with the wavelength maximum in each case at 340 mμ. The sulphuric acid chromogens produced by aliquots of the two solutions were also identical and exhibited absorption maxima at 285 and 430 mμ as compared to 285, 370, and 446 mμ for authentic 11-desoxycorticosterone. Substance 6B and authentic 17α-hydroxyprogesterone were each treated with 20 mg of sodium borohydride in 0.5 ml of 80% aqueous *tert*-butanol for 18 hours (10). The mixtures were then shaken with 1 g of sodium bismuthate in 4 ml of 50% aqueous acetic acid for 1 hour. The steroids were extracted as described above and chromatographed in the benzene:hexane:methanol:water solvent system. Substance 6B and 17α-hydroxyprogesterone both gave Δ^4-androstene-3,17-dione as the major product as well as three minor Zimmermann-positive substances with R_f values of 0.03, 0.17, and 0.35.

Substance 3A

The strip corresponding to authentic aldosterone from 2160 ml of plasma was applied as a spot and run in the toluene – propylene glycol system for 48 hours. There was no evidence for an ultraviolet-absorbing substance on the paper. The area corresponding to authentic aldosterone was cut out and extracted as described above. There was a negligible residue when the solvent was removed and when the fraction was run against an appropriate blank, there was no absorption maximum near 240 mμ. However, there was a shoulder in this region but if the total absorption was due to aldosterone it would represent less than 2 μg. The sample was taken up in ethanol and divided among six 5-day adrenalectomized rats. Three additional groups of eight rats were treated with 1, 4, or 16 μg of desoxycorticosterone acetate. The index of activity was the Na/K ratio in the urine sample collected 0–5 hours after injection. The sample proved to be virtually inactive giving a response less than that obtained with 1 μg of 11-desoxycorticosterone (DOCA). A sample of free aldosterone was also included in the assay at a dose of 0.25 μg and gave the expected response, with a potency of approximately 50$\times$DOCA (22).

Quantitative Determination of Steroids in Adams River Sockeye Plasma

Ethyl acetate extracts were prepared from 100-ml samples of the plasmas listed in Table I. The only modification from the large-scale isolation was in

TABLE I

Cortisone and cortisol in Adams River sockeye plasma

Date	No. fish	Location	Sex	Cortisone,* μg/100 ml	Cortisol,* μg/100 ml	Cortisol,† μg/100 ml	Total 17-hydroxysteroids, μg/100 ml‡
Oct. 16/58	34	Lower Fraser	M	—	—	8	—
Oct. 18/58	32	Lower Fraser	F	15	19	28	50
Oct. 27/58	21	Lower Fraser	M	27	12	11	33
	57	Lower Fraser	F	19	21	26	112 (62)§
Oct. 29/58	80	Lytton, B. C.	M	22	11	11	30
	80	Lytton, B. C.	F	41	26	24	126
Nov. 2/58	55	Adams Lake	M	27	64	66	154 (109)§
	15	"Spawned"	F	44	129	141	231

*Ethyl acetate plasma extracts purified by chromatography in chloroform:benzene 1:1 – formamide and toluene – propylene glycol and the steroids measured by the Porter–Silber reaction.

†Chloroform plasma extracts purified by chromatography in toluene – propylene glycol and the steroid measured by acid fluorescence.

‡Steroids determined directly on chloroform extracts of plasma with the Porter–Silber reagent.

§Because the Porter–Silber chromogen values did not agree with the sum of the cortisone and cortisol when the steroid levels were high, these samples were also assayed by the Eik-Nes modification of the Porter-Silber procedure (40).

the extraction of the 70% methanol fraction after the hexane partition. Three volumes of water were added to the 70% methanol and the steroids were extracted three times with 200-ml portions of chloroform. Each residue was then placed on a 14-cm length of prewashed Whatman No. 1 paper impregnated with formamide, and benzene:chloroform 1:1 was used as the developer. Cortisol, cortisone, and 17α-hydroxyprogesterone were run on an adjacent length of paper. The material from the plasma zone corresponding in position to authentic cortisol and cortisone was eluted from the paper as described above and run for 48 hours in the toluene – propylene glycol system. The areas of the plasma chromatogram corresponding to cortisone and cortisol were extracted from the paper, and made up to a suitable volume for analysis. Cortisol was analyzed both by acid fluorescence and the Porter–Silber reaction and the average taken while cortisone was analyzed by the latter method. The results are shown in Table I. Recovery experiments were carried out in duplicate with authentic standards which were added to 100-ml samples of Lytton male plasma at levels of 15 and 30 μg per 100 ml. The recovery of added cortisone through the entire procedure varied from 64 to 68% and averaged 65%. The recovery of added cortisol averaged 50%.

Quantitative Determination of Steroids in Prespawning Stuart Lake Sockeye Plasma

The plasma was pooled to give four samples from males and four from females (Table II). To 10 ml of each plasma 0.5 ml of 1 *N* sodium hydroxide was added and the mixture shaken and then extracted three times with 20-ml portions of chloroform (2). The chloroform extracts were chilled at 4° C overnight and filtered through glass wool. The chloroform was removed under nitrogen and the residue chromatographed in toluene – propylene glycol for 48 hours. The

TABLE II

Cortisol in Stuart Lake sockeye plasma

Location	Sex	No. fish	Cortisol, μg/100 ml	Sex	No. fish	Cortisol, μg/100 ml
Lillooet	M	10	18	F	10	42
Lillooet	M	10	27	F	10	37
Lillooet	M	10	18	F	10	37
Lillooet	M	10	21	F	—	—
			Av. 23			Av. 39
Forfar Creek	M	10	9	F	14	22
Forfar Creek	M	5	16	F	14	31
Forfar Creek	M	6	13	F	13	40
Forfar Creek	M	4	11	F	14	28
			Av. 13			Av. 30

area of the paper containing the cortisol was extracted with hot methanol and suitable aliquots were taken for acid fluorescence measurements.

The recovery of cortisol added to two of the samples treated as above was 65%. The results corrected for recovery are shown in Table II.

Discussion

The salmon pituitary has been shown to elaborate a substance with ACTH activity (23) and this gave rise to the hypothesis that the degenerative changes following spawning might be due to intolerable concentrations of adrenal cortical hormones produced under the influence of large amounts of ACTH (24). These studies have now demonstrated that Pacific salmon show extensive degeneration of the pituitary gland at full maturity and spawning, and this is accompanied by marked hyperplasia (and presumed increased secretory activity) of the adrenal gland. In contrast, mature rainbow trout, which spawn repeatedly, generally show little, if any, pituitary degeneration (25, 26, 27).

The greatly increased cortisol concentration in the plasma of both sexes following spawning is apparent by both chromatographic procedures, whether the final analysis was carried out by the Porter–Silber or the acid fluorescence method (Table I). The total 17-hydroxycorticosteroid levels as determined directly on chloroform extracts of plasma confirmed the greatly increased steroid content of the plasma following spawning. These data support the suggested high secretory activity of the adrenal gland at this stage in the life cycle. It appears plausible that these extremely high plasma hormone levels may play a role in the degenerative changes and death of the fish after spawning. Towards the completion of the present investigation a report appeared stating that several biochemical changes occurring in the plasma of an unidentified species of spawning Pacific salmon were consistent with adrenal hypercorticalism (28).

The presence of detectable quantities of cortisone was unexpected but even more surprising was the finding that it was a major plasma steroid in prespawning fish and predominated in several samples. Cortisol is the principal

plasma steroid in both sexes after completion of the spawning act. The data in Table I do not answer the one important question whether the increased cortisol level is coincident with the completion of the spawning act or whether there was a very significant increase between Lytton and spawning, a period of 13–15 days. Plasma samples were available from fish captured on arrival at the Stuart Lake spawning grounds (11). These fish were due to spawn in 4–5 days. Male plasma (100 ml) averaged 13 μg (Table II) as compared with 11 μg and female plasma averaged 30 μg as compared with 26 μg at Forfar Creek and Lytton respectively. These data suggest that there was a very sharp increase in the cortisol concentration in sockeye plasma during the brief period immediately preceding spawning and the completion of the spawning act. There appeared to be some decrease in plasma cortisol between Lillooet and Forfar Creek for the Stuart Lake sockeye. However, it should be emphasized that these samples were obtained in 1957 and, while they were stored at low temperature, some protein denaturation had occurred.

It is probably desirable at this time to interpret data on the Stuart Lake samples only to the point of indicating the order of magnitude of the cortisol concentration in plasma. The values may not be precisely comparable with those for the Adams River samples for the analyses of which the techniques were improved.

The total 17-hydroxysteroid levels are in most instances considerably higher than the combined cortisol and cortisone concentrations. There are many factors possibly contributing to this result, among which may be mentioned the contribution of 17-hydroxyprogesterone and the possible presence of non-steroid substances in the chloroform extracts of salmon plasma which react with phenylhydrazine. It has not yet been established whether the two steroids which accompany cortisol in the chloroform:benzene 1:1 – formamide system but separate from it in toluene – propylene glycol react with the Porter–Silber reagent. In any event, the total 17-hydroxycorticoid levels in human plasma have generally been found to be higher than the cortisol level even following chromatographic purification of the chloroform extracts (2).

The levels of cortisone and cortisol or the total 17-hydroxycorticosteroid levels found in the spawned fish or the total 17-hydroxycorticosteroid levels found in nearly all the female samples in the present study are sufficiently high to account for the glycogenic activity found in *Salmo salar* plasma (5). The report of this study was too abbreviated to permit an assessment of the possible reasons why the biological assay did not agree with the chemical (Porter–Silber) method. One conceivable explanation is that the less polar cortisone was not retained on the adsorbent along with the cortisol.

Another very important process which might be expected to be under hormone control during the spawning migration is the maintenance of electrolyte balance during and after the transition from salt to fresh water. Aldosterone is the most potent electrolyte-regulating hormone secreted by the human adrenal cortex. Aldosterone has been identified in the adrenal vein blood of hypophysectomized dogs (29). The level in normal human systemic blood is reported to be 0.08 μg 100 ml (30) and others report that sheep blood contains

0.35 μg/100 ml (31). Since aldosterone has a biological potency approximately 50 times that of 11-desoxycorticosterone in the assay method used in the present study, it would be expected that ca. 0.02–0.04 μg in the 2160-ml plasma sample could have been detected. If aldosterone were present at a concentration comparable with that in human plasma, approximately 1.7 μg should have been present, or 0.8–0.9 μg allowing for ca. 50% recovery.

Corticosterone has been reported to be the principal steroid in the circulating plasma of the rat (32) and is probably present at a level of approximately one-fifth that of cortisol in normal human plasma (8). However, only a very low concentration of corticosterone appears to be present in the female sockeye salmon plasma used in the present study. Both male and female sockeye salmon captured 415 miles from the spawning ground at Lillooet, B.C., on the 1956 Stuart Lake run and at Forfar, B. C. (Table II), were worked up as described in the experimental section and run in the toluene – propylene glycol system. The areas corresponding to authentic corticosterone were extracted from the paper and assayed by the fairly specific acid fluorescence method. The level of corticosterone was less than 1 μg/100 ml. Although it will be necessary to carry out studies on more samples before it can be concluded that the concentration of corticosterone is always extremely low in salmon plasma, it is highly unlikely that corticosterone plays a significant role in regulating ion balance. Another potent mineral-regulating hormone, 11-desoxycorticosterone, does not appear to be present in detectable quantities in human plasma or in the arterial blood of the dog. Small amounts (1.86 μg/100 ml) have been demonstrated in the adrenal blood of the dog (22). In the present study there was no evidence for the occurrence of 11-desoxycorticosterone in the large sample (1760 ml) of plasma. Therefore, none of the more probable electrolyte-regulating hormones was present in significant quantity in this salmon plasma.

Because of the relatively high levels of cortisol as compared with aldosterone in normal human plasma, it has been suggested that cortisol may play a significant role in maintaining normal mineral metabolism (30). If this conclusion is valid for human plasma, then it is indeed possible that the extremely high levels of cortisone and cortisol regulate mineral metabolism in salmon at least during the river migration.

The properties of substance 6B strongly suggest that it is identical with 17α-hydroxyprogesterone. The authors believe that this is the first reported isolation of this steroid from blood. The physiological effects of 17α-hydroxyprogesterone have only recently been investigated and a brief review may suggest some further line of research to the reader.

The efficacy of 17α-hydroxyprogesterone caproate and other progestogenic compounds has recently been discussed in detail (33). It is reported that 17α-hydroxyprogesterone has 60 times the progestational activity of progesterone in the Hooker-Forbes assay using Rockland-Swiss mice (34) but this compound gave negative results with the CHI strain (35). The progestational activity of 17α-hydroxyprogesterone in the Clauberg test was demonstrated to be increased greatly by esterification to the caproate (36). However, both 17α-hydroxyprogesterone and the caproate were significantly less active than

progesterone in the rat and mouse deciduoma and chick oviduct assays (37). The progestational activity of 17α-hydroxyprogesterone was reported to be much lower than that of progesterone when rabbits were the experimental animal. Neither 17α-hydroxyprogesterone nor the caproate exhibited significant glucocorticoid activity (38).

Progesterone has been measured in human placental blood and its metabolites Δ^4-pregnen-3-on,20β-ol and Δ^4-pregnen-3-on,20α-ol have been demonstrated in the human placenta (35). Progesterone-like activity has been reported in the plasma of oviparous snakes and it was suggested that its physiological importance was probably quite different from that of progesterone in mammals. The available evidence suggests that it is not concerned with gestation. However, there was an increase during gestation but the blood of the male with fully developed testes showed increased plasma levels (39).

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Addendum

As this paper was going to press, a report appeared, Hane, S. and Robertson, O. H. Proc. Natl. Acad. Sci. **45**, 886 (1959), on the Porter–Silber chromogen levels in king salmon plasma. These workers state that the ratio of cortisol to cortisone found by us for prespawning sockeye salmon was the reverse of that found for spawned king salmon. The present report shows that this is not a species difference but that the ratio of cortisol to cortisone found at Lytton does reverse following spawning. Hane and Robertson found no increase in the Porter–Silber chromogen material of plasma from spawned males. By contrast, the male sockeye examined in the present report showed both an increased Porter–Silber chromogen and cortisol concentration in the plasma.

PRINCE EDWARD ISLAND TROUT FISHERY*

by

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The waters of Prince Edward Island support good populations of brook trout. This species is indigenous to the area. The rainbow trout has been introduced and is establishing itself in an increasing number of streams.

Trout are anadromous

Both the native brook and the introduced rainbow trout run from Island streams into salt water. The movements of the trout between fresh and salt water present problems in production and utilization of the fish. Certain generalizations with respect to the anadromous behaviour of the brook trout can be made. The major movements between fresh and salt water occur in spring and early summer (April-June) and again in the fall and early winter (October-January). Within these seasonal periods, time of movements and numbers of trout involved vary from year to year. Temperature of water in the streams and estuarial waters, change in levels of water in the streams, size, age and maturity of the fish are factors affecting the movements. Trout may move simultaneously into and out of streams. In majority, trout first run from the streams when in their third year of age. Their stay in salt water varies greatly with the individual, from a few days to several months. In general, the trout are moving to larger living quarters with increase in size, and/or into waters of preferred temperature. There is no evidence

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* From a booklet prepared for the Prince Edward Island Department of Industry and Natural Resources and published in the Charlottetown Guardian of April 1, 1958, pp. 9-11

that the trout that run to salt water are a separate "race".

Brook trout are short-lived

Data on the age composition of Island brook trout populations show that the majority of the fish are short-lived. Trout over four years of age comprise less than five per cent of the trout that are of suitable angling size. Natural rather than angling mortalities largely determine this situation. Best use of the trout will be realized with as effective cropping as possible when the fish attain a satisfactory size to the majority of anglers.

Levels of trout production

The general angling success in Prince Edward Island streams and ponds indicates a high trout-producing capacity for the waters. Data on yields of trout to anglers and estimates of standing crops corroborate this view. During recent years anglers have taken an average of 1,750 trout (1.5 per hour) annually from the Ellerslie Brook system (5 miles long). A 23-acre pond has yielded (1943-1954) an average per year of 3,660 trout to the anglers (37 lb. per acre). Standing crops of trout in several ponds (as assessed by draining) have been found to vary considerably, but have been at the high level of 50 to 120 pounds per acre. For comparison, the yield of trout to anglers from eight less productive Charlotte County lakes, New Brunswick, was found to be in average 0.6 lb. per acre.

Studies of survival and growth with increasing densities of stock suggest that trout production is potentially higher than at present generally realized. Analyses of food supplies and their consumption by trout show that the food supplies are far from being fully utilized.

Factors conditioning good trout production

1. Character of drainage. The soils of Prince Edward Island are predominantly sandy and clayey loams. Because of the

nature of the soils and their development in depth, springs and spring seepages are widespread. These result in a good flow of cool water in most streams throughout the summer. The cool aquatic climate in the streams satisfies the temperature preferences of the trout at that season. Spring drainage provides favourable conditions for successful spawning.

2. Fertility of the waters. The drainage waters are well mineralized. A good part of the Island is intensively farmed. Fertilization of the soils enhances the supply of plant nutrients (nitrates, phosphates, potassium) in the drainage waters. There results a good growth of aquatic plants and trout foods (immature insects, snails, etc.) in the streams and ponds.

3. Restricted fish fauna in the fresh waters. Because it is an island and because of the character of the streams, the fish fauna of Island streams is restricted to a few species. Almost entirely the stream fish fauna consists of brook trout (dominant), young Atlantic salmon (subdominant) and the introduced rainbow trout (increasing numbers). Interspecific competition and predation are thus almost entirely limited to that between these three salmonoid species. In ponds formed on streams eels, sticklebacks and killifish may become common and occasionally abundant. In the estuaries the trout are of course associated with a greater number of fish species.

4. Estuaries. A common feature of the Island topography is the saltwater estuaries running inland at the mouths of freshwater streams. Brook and rainbow trout run from the streams into the estuaries. As gauged from recaptures of tagged individuals, most sea-run brook trout do not leave the estuaries for the open sea. The freshwater streams and the contiguous estuaries are favourable habitat units for the production of large numbers of trout.

5. Stream cover. A majority of the small streams provide a good cover for trout--logs and stumps, undercut banks and overhanging alders and other shrubbery. Cover is an important favourable factor in the production of brook trout.

Adverse conditions in the fresh waters for trout production

1. Deficiency in sizable natural fresh waters. Prince Edward Island, because of its small size, topography and character of soils, is an area of small streams. There are few natural ponds. The streams are suitable for the spawning of trout and provide favourable nurseries for young trout. However, there is a deficiency in sizable natural fresh waters to hold and grow the larger trout.

2. Silting. Much silt is washed into Island streams from farms. When slackened flow permits, silting of stream bottoms creates unfavourable conditions for trout. Lower reaches of streams and head of estuaries are often heavily silted. Local heavy silting may also arise by drainage from roadside ditches, particularly following new road construction. Silting presents a major problem in the maintenance of suitable depths for good trout production in ponds formed on streams.

3. Toxic plant sprays. Plant sprays which are toxic to fish are extensively employed in potato culture. Sporadically, wholesale destruction of trout results when poison is carelessly permitted to enter streams while filling and washing spraying apparatus and when discarding containers. Less discernible, but more widespread in effect, is possible damage from poisons washed into the streams from fields with sprayed crops. Observed poor populations of trout in seemingly suitable streams may have resulted in this manner.

4. Predation. Brook trout are cannibals. Predation may occur between brook trout, rainbow trout and salmon parr in the streams.

The great blue heron, kingfisher and mink are serious predators of trout. With decline in trapping of mink for fur, their numbers have become larger.

Conditions limiting utilization of trout by anglers

1. Poor accessibility of streams to anglers. Trees, alders and other shrubbery make it impossible for the fly-fishermen and difficult, if not also impossible, for the bait-fishermen to angle trout from long reaches of most Prince Edward Island streams. Sea-run trout, which constitute a good proportion of the trout of suitable angling size, are most accessible to anglers at the heads of estuaries and along the more open sections of streams. However, the majority of the sea-run trout on their return to the streams move through such areas, unless barred by obstructions, into the more covered and less accessible upstream stretches of the streams before they can be taken by anglers. Natural mortalities there account for large numbers of these fish and they are thus lost to the angler.

It is to be emphasized, however, that the same cover that deters angling does much to condition the high trout-producing capacities of the streams. Cover should be viewed as a favourable rather than an unfavourable factor in the management of the Island trout fishery, and should not be purposely altered in any extensive way. Rather it would appear more practical to hold the sea-run trout at accessible points to make them more obtainable by the anglers.

2. Plant growths. The fertility of Prince Edward Island waters that makes basically for good trout production is not an unmixed blessing. Filamentous algae ("scum") and rooted aquatic

vegetation often grow profusely in ponds formed on streams. By midsummer these plant growths virtually prevent angling in some ponds by fouling of the fishing hooks.

Improvement of the trout fishery

Improvement of the Island trout fishery can be realized (A) by improving the utilization of existing stocks of trout, and (B) by improving the production of young and older trout.

(A) Improvement of utilization

1. Pond formation on streams. Pond formation is viewed as a method of making trout readily available to anglers. The view is based on the angling success enjoyed in most Island ponds formed on streams. However, dams curtail the movements of trout, particularly between fresh and salt water. Sea-run trout are highly regarded by anglers. To evaluate the effectiveness of pond formation, information of the following sorts is needed:

(i) the effects of pond formation on the movements of trout between fresh and salt water;

(ii) the capacity of ponds to produce trout;

(iii) the yield of trout to anglers from ponds;

(iv) the comparative yield of trout to anglers from a stream system--stream, pond, estuary --before and after pond formation.

Investigation of the Island trout fishery has provided pertinent information which may be summarized as follows:

(i) The number of trout running to salt water is reduced by pond formation. The pond assumes the function of the estuary in varying degrees and holds trout in fresh water, although they are free to escape over the dam. Where the

stream is small and the supply of young trout small in relation to size of the pond formed, the movement of salt water is markedly reduced. This occurs to a less degree in the situation where the stream is larger and the supply of young trout greater.

(ii) Ponds formed on Island streams are highly productive of trout.

(iii) Annual yields of trout to anglers from such ponds is high.

(iv) When closely studied (Ellerslie Brook) pond formation resulted in an average increase of 40 per cent in the number of trout annually taken from the stream system.

The data warrant the recommendation of pond formation as a management procedure to improve the yield of trout to anglers. This recommendation will need review in the future if pond formation should become so widely applied as to affect materially the supply of sea-run trout in the Island area.

To be most effective as a trout-producing area, it is recommended that the following actions be taken in forming a pond:

(i) that the pond be as large as circumstances permit and not less than one acre in area;

(ii) that a depth of six to ten feet of water be realized over at least 50 per cent of the area;

(iii) that the dam be constructed so that the pond can be drained to the original stream level to correct adverse effects of silting, to control growth of rooted aquatic vegetation and to control numbers of fish;

(iv) that provision be made in the construction of the dam for screening to prevent exit of trout.

Screening would be desirable when the supply of trout is below the trout-producing capacity of the pond, but undesirable when the supply is sufficiently large to create crowding and stunting of growth.

2. Control of up stream movements of sea-run trout. The majority of sea-run trout, on their return to the streams, move into sections where they are poorly accessible to the anglers. From tagging and recapture, it is shown that a large proportion of these trout die naturally in the streams and are poorly cropped by the fishermen. Pools below dams are well appreciated as excellent fishing spots for sea-run trout. By remaining in such pools during the summer, the sea-run trout are more effectively cropped than if they had had the opportunity to disperse into the upper reaches of the stream. These observations point to a procedure of erecting barriers at or near the mouths of streams for holding the sea-run trout and making them more available to the anglers. It is recommended that barriers be constructed on a few streams on a trial basis for this purpose.

The barriers may be fences constructed of panels made of laths or metal rods spaced three quarters of an inch apart, or low dams. It is a prerequisite that there be a pool, or one created, of five feet or more in depth immediately, or a short distance below the barrier. (It has been observed that appreciable numbers of trout are held in pools below dams only when the pools are several feet in depth.)

It is recommended that the barriers be temporary, only functioning to prevent the up stream (and downstream) movement of the trout between mid May and late September. Such barriers would principally affect the up stream June-July ("strawberry") runs of sea-trout. They would leave unaffected the important up- and downstream runs of trout that occur in the fall after the angling season. In any event, barriers would be difficult to maintain in the heavy waters of spring and fall, but relatively easy to hold during the summer season.

3. Control of aquatic plant growths in ponds. Algae ("scum") and other aquatic plants are killed by low concentrations of copper sulphate and sodium arsenite, or mixtures of the two chemicals. Effective concentration depends upon the hardness of the water and the quantity of plant growth. Thus it has to be gauged largely by assessment of local conditions. Control is temporary.

The above chemicals are also toxic to fish, but usually at somewhat higher concentrations than necessary to destroy aquatic plants. However, the margin of safety is small, and no general prescription can be given with assurance of not killing trout. Use of these chemicals to control plants in Island ponds without killing trout can be recommended only under strict supervision, after effective concentrations for local situations have been determined.

4. New technique for angling. Trolling for trout with spinners, etc., from boats is a common method of fishing in New Brunswick and Nova Scotia lakes. It is not practised in Prince Edward Island waters but might very well prove effective in estuaries particularly, and in the larger ponds. Demonstration of the technique is needed.

(B) Improvement in trout production

It is advocated that immediate improvement in trout angling can be obtained by improving the accessibility of trout to anglers, and thus realize a fuller, more efficient cropping of the good population of trout that now exists in Island waters. It would be short-sighted to assume, however, that the future and greater demand for trout will be satisfied in this manner without hand-in-hand improvement of the production of trout.

1. Pond formation on streams. The production of trout in Island ponds has been studied by assessing the growth and survival with varying, but known densities of stock. Results show that ponds formed on Island streams are highly

productive of trout. Determined yields of trout from ponds (weight removed less weight introduced) over summer periods have been as high as 50 pounds per acre. Ponds not only increase the yield of trout to anglers by making the fish more accessible but also materially increase the supply as a result of their high productive levels. The recommendation of pond formation as a management procedure to improve angling envisages that ponds improve both accessibility and production of trout.

2. Control of silting. The soils of Prince Edward Island are easily eroded. Reduction in the silting of streams, ponds and estuaries can only be realized and will be directly commensurate with the extent of application of agricultural procedures, such as contour plowing, which lessens erosion of farm lands. Severe local silting which often accompanies road construction can often be materially reduced by having tap drains from road ditches terminate in low lying areas of land rather than directly in the nearest pond or stream.

3. Other procedures. Stream improvement (pool formation, increasing shelter), stocking of hatchery-reared trout, predator control, control of intra- and interspecific competition between fish, fertilization of the waters, artificial feeding of fish are procedures which have proved helpful in varying degrees in other fresh waters for improved production of trout. Opinion on their effectiveness in Island fresh waters is reserved until further data are available. Compared to most fresh waters the natural production of trout on the Island is high. The question to be answered is not so much whether these procedures will improve trout production as whether they will do so to a substantial and worthwhile degree, and add significantly to the existing high level of production.

North (Yorke) River Pond

As a result of building a causeway to carry the Trans-Canada highway across the North or Yorke River near

Charlottetown, a large pond of about 500 acres was created (1955) on what was formerly a tidal estuary. This empoundment is unique for the Island area because of its size and because it is the empoundment of an estuary.

Salt water can enter the empoundment at high tide via a sluit through the causeway. Inflow of salt water can be controlled by a valve and flap-gates installed at the pond end of the sluit.

Hydrographic conditions in the empoundment were observed in 1956 and 1957, when salt water was permitted to enter. In both years the waters were sharply stratified with respect to salinity and temperature in summer. Decomposition of organic matter in the salt water, stagnated below about nine feet in depth, resulted in a depletion of dissolved oxygen (necessary for fish life). There was thus a large volume of the water in the pond which was uninhabitable to trout because of the lack of dissolved oxygen, but, with respect to temperature of water, quite favourable for the species. At the same time in the summer the surface waters contained sufficient oxygen for trout but they were too warm. In total, therefore, much the greater volume of water in the pond was unfavourable for trout.

Trout were netted from the empoundment in the summers of 1956 and 1957. However, they were found only in a shallow layer of suitable water wedged between the lower oxygen-deficient strata and the warm surface waters.

The pond is viewed as potentially a good trout-producing area. Unfavourable conditions for trout result largely from the empoundment and stagnation of salt water. It has been recommended that the inflow of salt water be stopped. It is anticipated that the saline oxygen-deficient waters will be replaced by those which are fresh sufficiently cool and aerated in summer for trout. Continued observation is needed to learn how soon and how well the anticipated events will occur with exclusion of salt water.

With exclusion of salt water, sea-run trout will not be able to re-enter the pond. The pond is fed by a number of spring-fed streams, with a combined drainage area of about 50 square miles. The streams are well populated with young trout. Both supply of water and of young trout for the pond would appear to be assured at a good level.

Development of the Island trout fishery

The trout as a natural asset of the Island is being increasingly appreciated. However, this asset is as yet far from being fully exploited. As with any natural asset, development of the trout fishery requires investment.

The trout fishing is an attraction to the tourist. Angling success is and will be the criterion by which the tourists evaluate the Island trout fishery. Greater assurance of angling success during July and August is needed to enhance the trout angling as an attraction to the tourists. To do this, the following scheme is proposed.

The scheme involves ponds which the Province may control or secure control of, or better, create with those characteristics known to give the most favourable trout production. With the present angling season (April 15 to September 15) a good proportion of the annual crop of trout from ponds is removed by resident anglers in the spring before the arrival of the tourists. It is proposed that a number of ponds controlled by the Province be closed to angling until July and August, and then opened to both resident and tourist fishermen. This procedure would assure better angling for the tourists, and, since attention would be drawn to specific ponds, effective utilization of the resource would also be realized.

Closure of ponds until July or August would necessitate guardianship. It is suggested that the owner of the land on which the ponds are situated be paid an annual retainer to

serve as guardian. It is further suggested that such land-owners be encouraged to provide boats for hire, thereby assuring facilities for angling and also giving revenue to the owner.

It is considered that the value of this procedure as a method of attracting tourists should first be demonstrated by the Province, or under other public auspices. With demonstration, private enterprise would be stimulated. Profit on private investment could be realized from the sale of angling privileges (rod fees, charge for fish caught). Trout farming for this purpose or for sale of trout as a luxury food item, has unexplored possibilities on Prince Edward Island, where high natural productive levels for trout demonstrate the suitability of the waters.

It should be noted that some of the proposed schemes involving erecting barriers in streams, poisoning waters, and closed seasons require specific authorization of the Federal Department of Fisheries.

Report on Trimethylamine in Fish

By W. J. DYER, *Associate Referee* (Fisheries Research Board of Canada, Technological Station, Halifax, Nova Scotia, Canada)

The Associate Referee undertook an investigation of methods for the determination of trimethylamine in an attempt to find a suitable procedure for its detection in fish and fish products. Any method that seemed promising would be studied further to ascertain its accuracy and precision, especially under routine analytical practice.

Two quite different procedures are presently in use: the Conway procedure of Beatty and Gibbons (1) and the colorimetric procedure of Dyer (2).

To be of value to the fishing industry, any method can be considered useful only if it is adaptable to routine use on large samples. Therefore only the Dyer procedure was studied.

For food inspection purposes, that is, to support judgments of fitness for food, the sensitivity and range of the present procedure is satisfactory. If the method is to be used to check grading of fish of nearly top quality, however, the sensitivity should be increased.

Several extraction procedures have been proposed:

(*a*) Trichloroacetic acid was used in the original procedure (3). A 100 g sample of fish (a piece of fillet, or a minced sample well mixed, with minimum loss of drip), was blended with 200 ml 7.5% trichloroacetic acid for a few minutes. (The extract may be pipetted directly after settling or it may be centrifuged or filtered.) The extract is stable.

(*b*) In Shewan's method (private communication) 5 grams of solid trichloroacetic acid is added to 100 grams of fish. This necessitates expressing values directly on the extract or applying a correction to obtain values based on sample weight.

(*c*) In the Dyer and Mounsey method (3) 100 grams of fish is blended with 200 ml of 5% formaldehyde solution (1 volume of commercial HCHO diluted to 8 volumes with water). The extract is stable.

(*d*) In Hoogland's method (4) 100 grams of fish is blended with 200 ml of water. The extract is not stable; although it is satisfactory for cod and haddock, some reports indicate that extraction of certain other species is incomplete. Troublesome emulsions also may be formed in the later solvent extractions.

Methods (*a*) and (*c*), and in most cases method (*d*), give similar results.

For official purposes, trichloroacetic acid is probably the most satisfactory extractant, but a less expensive reagent is desirable for routine work. Further study should be undertaken either to improve the water procedure or to find a less obnoxious reagent than formaldehyde.

The basic colorimetric procedure used here was that of Dyer (2, 5). The extraction procedure included the use of trichloroacetic acid (3). To check the colorimetric estimation of trimethylamine hydrochloride standards, samples of unknown concentration were submitted to an analytical laboratory, an industrial control laboratory, and an inspection control laboratory.

METHOD

Reagents

(a) *Trichloroacetic acid.*—7.5% aqueous solution.

(b) *Toluene.*—Reagent grade, dried over anhydrous Na_2SO_4. To remove interferences, shake with 1*N* H_2SO_4, distill, and dry with anhydrous Na_2SO_4.

(c) *Picric acid.*—Stock solution: Dissolve 2 g dry picric acid, reagent grade, in 100 ml moisture-free toluene. Working solution: Dilute 1 ml to 100 ml with moisture-free toluene.

(d) *Potassium carbonate solution.*—Dissolve 100 g K_2CO_3 in 100 g water.

(e) *Formaldehyde.*—Dilute 10 ml formalin (40% formaldehyde, commercial, shaken with magnesium carbonate, and filtered) to 100 ml with water.

(f) *Trimethylamine standard solution.*—Stock solution: Add 0.682 g trimethylamine hydrochloride to 1 ml HCl and dilute to 100 ml with

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Table I. Analysis of standard samples of trimethylamine hydrochloride

Sample	Trimethylamine Present[a]	Trimethylamine Found[b] Lab. I	Lab. II	Lab. III
A	0.367	0.37(±0.01)(4)	0.32(±0.08)(10)	0.35(±0.01)(3)
B	2.22	2.19(±0.06)(4)	2.2 (±0.12)(7)	2.30(±0.02)(3)
C	6.61	6.46(±0.29)(4)	6.3 (±0.14)(15)	6.70(±0.09)(3)

[a] Amounts of trimethylamine hydrochloride present in the solution were equivalent to the mg trimethylamine nitrogen per 100 g fish shown.
[b] Last number in parentheses after standard deviation is number of determinations reported.

water. Check basic nitrogen content of 5 ml aliquots by adding alkali and distilling into standard acid in a micro-Kjeldahl distillation apparatus. This solution is quite stable. Dilute standard: add 1 ml to 1 ml HCl and dilute to 100 ml with water.

Determination

(a) *Extraction.*—Weigh 100 g minced or chopped, well mixed sample of fish or fillet. Add 200 ml 7.5% trichloroacetic acid, and blend, or if more convenient, shake occasionally over a period of several hours. (The mixture is stable and often is used without filtration.)

(b) *Colorimetric estimation.*—Pipet an aliquot (preferably containing 0.01–0.03 mg trimethylamine nitrogen) into a 20 × 150 mm Pyrex test tube. For trimethylamine values (mg N per 100 g) in the range 1–5 use 1 ml extract and dilute with water to 4.0 ml. Add 1 ml HCHO reagent, 10 ml toluene from an automatic pipet, and 3 ml K_2CO_3 solution. Stopper the tube with a polyethylene stopper and shake vigorously by hand about 40 times. Pipet off 5 ml of the toluene layer into a small test tube containing about 0.3 g anhydrous granular Na_2SO_4. Avoid removing droplets of the aqueous layer. Stopper the tube with a polyethylene stopper and shake gently a few times to dry the toluene. Decant into a dry colorimeter tube. Add 5 ml picric acid reagent and mix by swirling gently. Read in a colorimeter, using a filter with maximum transmittance at 410 mμ against a blank carried through the procedure. The color is stable. (The Evelyn 400 filter gives better results than the 420 filter.)

Notes.—Do not use stopcock grease; a mixture of sugar and glycerol ground together may be used if necessary. Do not wash the tubes with soap or detergent. Rinse them with water and occasionally clean them thoroughly with nitric acid.

Calculations.—Mg trimethylamine nitrogen in aliquot = absorbance/K, where K is a constant which varies according to the instrument used. To convert this value to mg N per 100 g fish, divide by the number of ml in the aliquot and multiply by the total volume of extract (300 ml).

Table 2. Deviation from amount of trimethylamine present (%)

Sample	Trimethylamine Present, mg N/100 g Fish	Deviation, % Lab. I	Lab. II	Lab. III
A	0.367	−0.8	−18	−4.1
B	2.22	−1.8	0	+3.4
C	6.61	−2.3	−4.7	+1.3
Av.		1.6	7.6	2.9

Results and Recommendation

Preliminary results obtained on standard solutions of trimethylamine are reported in Table 1. These results agree very well with the actual amounts present. The deviations are shown in Table 2 as percentages of the actual amount of trimethylamine present.

Table 3. Deviation from average trimethylamine (%)

Sample	Av. Trimethylamine Found, mg/100 g Fish	Deviation, % Lab. I	Lab. II	Lab. III	Av. % Dev. from Mean
A	0.35	+8.8	−5.9	+3.0	5.9
B	2.23	−1.8	−1.3	+3.1	2.1
C	6.49	−0.5	−2.9	+3.2	2.2
Av.		3.7	3.4	3.1	

The results for Sample A (very low in trimethylamine) are considered very good; they range from −0.8 to −18%. Sample B ranges only from −0.8 to +3.4% deviation from the amount added. Sample C is comparable to Sample B.

In Table 3 are shown the deviations from the mean results found for each sample. Again agreement is excellent.

The 17 results reported for Sample A

average 0.340 (±0.06) or ±19%; the 14 results for Sample B average 2.22 (±0.10) or ±4.5%; and the 22 results for Sample C average 6.36 (±0.23) or ±3.6%. Thus for trimethylamine values below 1, the results agreed within 20%; with higher values, they agreed within about 5%. These results indicate that the present procedure is satisfactory.

The Associate Referee plans a similar study on stable extracts from fish. It is probably not feasible to do collaborative analyses on the fish muscle itself because of its susceptibility to change.

It is recommended* that the method be studied on actual samples of fish extracts.

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The Direct Estimation of Ammonia in Sea Water with Notes on "Reactive" Iron, Nitrate, and Inorganic Phosphorus

By

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The Estimation of Ammonia

Dr. W. R. G. ATKINS in the June 1957 issue of this Journal (ATKINS, 1957) drew attention to the necessity for a rapid method for the direct determination of ammonia in sea water, a method not requiring distillation or other methods of separation. He pointed out the possibilities inherent in the pyrazolone technique originated by KRUSE and MELLON (KRUSE and MELLON, 1952 and 1953).

We have critically examined most of the published methods for the direct determination of ammonia in sea water. The procedure given by BULJAN (1951) held great promise but for reasons that we cannot understand this method was a complete failure in our hands. The Kruse and Mellon method, however, has been found to be applicable to sea water, with slight modifications, and appears to us to be superior in speed, accuracy, and precision to any other method so far described. A brief account of the technique will be given here and very complete working details may be obtained from *Standard Methods of Seawater Analysis*, Vol. 2 (STRICKLAND, 1958) obtainable from the Pacific Oceanographic Group, Nanaimo, B. C., Canada.

Reagents

De-ionized water should always be used, distilled water being passed through a small column of Dowex 50 or a similar resin in the hydrogen form immediately before use.

Carbon tetrachloride. Analytical reagent quality material is satisfactory.

Pyridine. Analytical reagent grade pyridine has been found to be satisfactory but this may not always be the case. Other sources are generally useless. The

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reagent should be distilled with a few drops of bromine, using a fractionating column, and collecting the fraction boiling in a 2–3°C range near to 113°C (uncorrected). Unsuitable pyridine is easily recognized because a strong blue-green colour develops in the sea water before extraction whereas little or no colour is found in the carbon tetrachloride layer (see below).

Bis – (3-methyl-1-phenyl-5-pyrazolone) (Bis reagent). This compound is obtainable as Eastman-Kodak 6969, 3-3′-dimethyl-1-1′-diphenyl-[$4_1$4′-bi-2-pyrazoline]-5-5′-dione or can easily be prepared as described by KRUSE and MELLON (1953).

Chloramine-T solution. 5% in de-ionized water, stable for not more than one to two weeks.

3-methyl-1-phenyl-5-pyrazolone solution (Mono reagent). Recrystallize this substance once from hot water, and prepare an 0·25% solution in de-ionized water. Stable indefinitely.

Pyrazolone reagent. Prepare immediately before use (storing for no longer than 1–2 hours), by dissolving 0·08 g of the Bis reagent in 80 ml of pyridine and then adding 400 ml of Mono reagent.

Acetate buffer. Dissolve 11·0 g of analytical reagent quality sodium acetate trihydrate in a little water, add 65 ml of analytical reagent quality glacial acetic acid and make to a volume of 500 ml with de-ionized water.

Method

Measure 80 ± 2 ml of sea-water sample, at a temperature between 18°C and 23°C into a 200 ml separating funnel, and add 1·0 ml of acetate buffer. Add 1·0 ml of chloramine-T solution, and wait for at least 90 seconds, although a period of up to 5 minutes does no harm. Add 40 ml of pyrazolone reagent and after at least 1 minute (5 minutes is still satisfactory) add 35 ± 0·5 ml of carbon tetrachloride from a dry 50 ml measuring cylinder. Extract the ammonia complex vigorously for at least one minute and allow the layers to separate. Run off the carbon tetrachloride layer through a small dry filter paper into a dry 10 cm spectrophotometer cell and measure the extinction against water at 4500 Å. The carbon tetrachloride extract is stable for at least 24 hours if evaporation is prevented.

Calculations

Sea-water salts reduce the extinction obtained from a given amount of ammonia. The extinction – salinity relationship is curved but between a salinity of 25‰ and 35‰ can be approximated to a straight line. The extinction obtained in pure water is some 1·6 times as great as the value obtained from the same amount of ammonia in water of a salinity of 30‰, much of the suppression occurring as the salinity increases from zero to about 15‰.

Each batch of samples should be corrected for a reagent blank by subtracting 0·62 times the extinction obtained from 80 ml of de-ionized water carried through the method at the same time as the samples. This blank extinction varies from batch to batch but is generally in the range 0·1 to 0·15 and appears to effect a satisfactory correction to each batch of samples. The final ammonia concentration may be calculated from the expression:

$$\mu g \text{ at. ammonia N/l} = \frac{\text{corrected extinction} \times 8{\cdot}5}{(1 - 7{\cdot}6 \times 10^{-3} \times S)}$$

where S is the salinity in $^0/_{00}$.

Little error is incurred with samples having a salinity between $29^0/_{00}$ and $34{\cdot}5^0/_{00}$ by neglecting the salinity correction and using a factor obtained by calibration with $30^0/_{00}$ sea water, viz:

$$\mu g \text{ at. ammonia N/l} = \text{corrected extinction} \times 11$$

Results

The method works very well in a shore-base laboratory, provided that the usual scrupulous precautions are taken against contamination, and has been used successfully at sea, working with batches of about 6 samples at a time (2 hours for complete analysis). The recovery of ammonium ion added to sea water is complete. The standard deviation of the method at a level of 5 μg at. N/l is about 0·2 μg at. and at 0·7 μg at. N/l is about 0·1 μg at. The limit of sure detection is around 0·1–0·2 μg at. N/l under normal routine working conditions. The surface coastal waters of the north-east Pacific in July 1958 had as much as 5 μg at. N/l of ammonia nitrogen present, but at a few metres depth the amount rarely exceeded 1–2 μg at. N/l. However, as much as 0·5–1 μg at. N/l was found at all depths down to 900 m, distributed in a somewhat erratic pattern. In this regard, the erratic results obtained by Cooper (1948a) should be borne in mind.

Notes on the Estimation of Reactive Iron

Goldberg, Baker, and Fox (1952) pointed out the value of membrane filters of approximately 0·5 micron pore size in differentiating between "particulate" and "soluble" iron in sea water and Lewis and Goldberg (1954) have described an excellent procedure for iron determinations using these membranes and α–α' dipyridyl for "particulate" and bathophenanthroline for "soluble" iron. For both analyses fuming perchloric acid was used to leach all forms of marine iron into true ionic solution. Recently Armstrong (1957) recommended an autoclave technique using approximately 0·14 M hydrochloric acid to bring iron into solution. A less extreme form of attack was used earlier by Cooper (1948).

Although these methods, certainly the one described by Lewis and Goldberg (1954), may bring about the dissolution of most forms of combined iron into a form reacting with 1:10 phenanthroline or similar reagents it is doubtful whether the results necessarily bear a close relationship to the amount of iron in sea water that is readily and immediately available to growing phytoplankton.

Harvey (1937) and more recently Goldberg (1952) have produced evidence that the iron readily and rapidly available to growing phytoplankton is most probably in the form of small particles of hydroxide or phosphate. "Soluble" iron is available if present as colloidal hydroxide but is probably of little use if strongly complexed by organic material. It seems reasonable, therefore, to sort both "particulate" and "soluble" iron into biologically reactive and unreactive forms by treatment with a reagent that will rapidly dissolve all forms of precipitated or colloidal ferric hydroxide but leave untouched material such as

ignited iron oxides, ferric silicate compounds, unreactive clays and muds, etc. The problem is kinetic rather than a matter of thermodynamics. Treatment with hot hydrochloric acid of about 0·5 M concentration for 10–15 minutes dissolves all forms of precipitated ferric hydroxide and phosphate, even material dried to 110°C, but is practically without effect on less reactive ferric materials such as ignited iron oxides and clays and appears to have no marked leaching effect on the iron in plant tissues. The use of such a preliminary treatment is of great assistance, analytically, as no evaporation and fuming with strong acid is required.

We suggest treating a millipore HA filter, holding the "particulate" iron from a suitable volume of sea water, with 10 ml of a solution of 20·0 ml concentrated hydrochloric acid (s. g. 1·18) in 500 ml of water. The filter is digested in this extraction reagent by placing it at the bottom of a 50 ml stoppered measuring cylinder and heating the cylinder for 10 minutes in a boiling, or near boiling, water bath. After cooling the cylinder 2·0 ml of a sodium acetate buffer (75 g of trihydrate dissolved in 100 ml of water) are added and the determination may then be continued using α–α' dipyridyl as described by Lewis and Goldberg (1954) or others. Similarly for "soluble" iron, 100 ml of sample is allowed to stand with 10 ml of hydrochloric acid extraction reagent for about 15 minutes. After adding 2·0 ml of acetate buffer the determination may be continued as described by Lewis and Goldberg (1954), except that we have found that one extraction with 30 ml of iso-amyl alcohol is sufficient to remove iron from the aqueous layer. Full working details of the proposed method may be obtained from the *Standard Methods of Seawater Analysis*, Vol. 2, referred to earlier.

It would be useless to suggest that the above procedure is anything but arbitrary. However, it appears reasonable to assume that the amount of iron brought into solution by this method bears a more direct relation to the amount of immediate use to growing plant cells than does the total iron content of a sea-water sample. Results by the proposed method in north-east Pacific oceanic and coastal waters indicated that the total reactive iron rarely exceeded 0·3 μg at. Fe/l, even at the surface, and that generally half or more of this iron was "soluble". Methods measuring total iron generally give several times more "particulate" iron than "soluble" iron.

Notes on the Estimation of Nitrate

The copper catalyzed hydrazine reduction of nitrate to nitrite described by Mullin and Riley (1955) gives rise to an elegant and convenient method for the direct determination of nitrate in sea water. The procedure has shown great promise in our hands but two important factors affecting the method have come to light and may be worth reporting.

The extinction for a given concentration of nitrate is a function of the size and shape of the glass vessel used for the reduction. About 50 ml of sea water in a 125 ml Erlenmeyer flask gives a sensitivity that is nearly double that found when using the 50 ml volumetric flasks recommended in the original method. For both convenience and sensitivity, therefore, we favour the use of Erlenmeyer flasks but a drawback is that the resulting extinction is then affected by any motion of the flasks and is reduced by the rocking experienced during shipboard analyses. This may be overcome by using a small serological water

bath in gimbals and by processing standards with every batch of samples analysed. Reductions carried out in 50 ml volumetric flasks, filled to the neck, are not sensitive to motion.

Although the sensitivity of the method is not a function of temperature, over a reasonable temperature range, as stated by the authors, we have found that it is sensitive to *changes* in temperature during the first few hours of reduction. Flasks should be incubated with reductant in a thermostatically controlled water bath, the temperature of which does not change by more than ± 1°C throughout the first 4–6 hours of reduction. A *rise or fall* in temperature during this period can cause a drop in extinction of as much as 10–30%. Provided that the temperature is held constant it may be anywhere in the range of about 18°C–25°C without affecting the sensitivity.

Notes on the Estimation of Phosphate

Recently MURPHY and RILEY (1958) have proposed a single solution method for the determination of inorganic phosphorus in sea water. Samples are treated with a mixed molybdate – sulphuric acid – ascorbic acid mixture which gives rise to a procedure which is practically as sensitive as those using stannous chloride as reductant but which has none of the disadvantages of time and temperature dependence; repeated standardizations are unnecessary.

However the necessary reduction time is over 20 hours at room temperature or some 30–60 minutes at 50°C, as compared with the relatively few minutes at room temperature needed with stannous chloride. Such a lengthy reaction time in a solution which is about 0·25 M with respect to sulphuric acid might reasonably be supposed to effect the hydrolysis of organic phosphorous compounds in sea water. The authors did not find this to be the case, but we have to report that serious errors may result if the method is used in areas, such as British Columbia coastal waters, where the soluble organic phosphorus content of the water often exceeds the inorganic orthophosphate content. At Departure Bay (Vancouver Island) an analysis of the water at 5 m depth in June 1958 showed the inorganic phosphate to be 1·03 ± 0·05 μg at. P l (stannous chloride), the soluble organic phosphorus to be 1·16 ± 0·11 μg at. P l and the particulate phosphorus to be 0·92 ± 0·13 μg at. P/l. (The limits indicate an uncertainty range at the 0·05 probability level.) Simultaneous analysis by the Murphy-Riley method gave an apparent inorganic phosphorus value of 1·41 ± 0·07 μg at. P l and it seems almost certain, therefore, that nearly a half of the soluble organic phosphorus compounds mineralized during the reduction.

Conditions here are probably extreme, and where the absence of organic phosphorus is ensured the ascorbic acid reduction technique has everything to recommend it. However, when a precise evaluation of the various forms of phosphorus is desired a reduction with stannous chloride still appears to be mandatory, despite the inconvenience, as a reduction in the shortest time at the lowest practicable temperature must be attempted.

Summary

The Kruse and Mellon extraction method for ammonia in sewage has been adapted for shipboard use in the analysis of sea water. Brief working details

are given. A method for the estimation of biologically reactive iron in sea water is suggested, based on the reactivity of marine iron compounds to dilute hydrochloric acid. Some notes are given on the use of the nitrate method of MULLINS and RILEY (1955) and on the "single solution" phosphate method of MURPHY and RILEY (1958).

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A RAPID METHOD OF TOTAL LIPID EXTRACTION AND PURIFICATION[1]

E. G. Bligh and W. J. Dyer

Abstract

Lipid decomposition studies in frozen fish have led to the development of a simple and rapid method for the extraction and purification of lipids from biological materials. The entire procedure can be carried out in approximately 10 minutes; it is efficient, reproducible, and free from deleterious manipulations. The wet tissue is homogenized with a mixture of chloroform and methanol in such proportions that a miscible system is formed with the water in the tissue. Dilution with chloroform and water separates the homogenate into two layers, the chloroform layer containing all the lipids and the methanolic layer containing all the non-lipids. A purified lipid extract is obtained merely by isolating the chloroform layer. The method has been applied to fish muscle and may easily be adapted to use with other tissues.

Introduction

In the course of investigations concerning the deterioration of lipids in frozen fish, the need arose for an efficient and rapid method of total lipid extraction and purification. Furthermore, due to the highly unsaturated nature of fish lipids, the method had to involve only mild treatment so as to minimize oxidative decomposition and the production of artifacts. Several existing methods were considered but none was entirely satisfactory. The methods of Dambergs (1) and Folch *et al.* (2) were too time-consuming for routine investigations and since the former method entailed heating and evaporation it was considered unsuitable for lipid composition studies. The recent method of Folch *et al.* (3), which was published while this study was in progress, was more rapid than their previous method but still had the disadvantage of employing large and inconvenient volumes of solvent. The method used by Dyer and Morton (4) was rapid but extracted only a fraction of the total lipid.

The present paper describes a method whereby the lipids of biological materials can be extracted and purified in a single operation.

Mixtures of chloroform and methanol have had wide use as lipid extractants and examination of the chloroform–methanol–water phase diagram (Fig. 1) led to the following hypothesis. Optimum lipid extraction should result when

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the tissue is homogenized with a mixture of chloroform and methanol which, when mixed with the water in the tissue, would yield a monophasic solution. The resulting homogenate could then be diluted with water and/or chloroform to produce a biphasic system, the chloroform layer of which should contain the lipids and the methanol–water layer the non-lipids. Hence, a purified lipid extract should be obtained when the chloroform layer is isolated.

Procedure

Reagents

Methanol, absolute, analytical reagent; chloroform, analytical reagent.

Lipid Extraction and Purification

The following procedure applies to tissues like cod muscle that contain $80 \pm 1\%$ water and about 1% lipid. Each 100-g sample of the fresh or frozen tissue is homogenized in a Waring Blendor for 2 minutes with a mixture of 100 ml chloroform and 200 ml methanol. To the mixture is then added 100 ml chloroform and after blending for 30 seconds, 100 ml distilled water is added and blending continued for another 30 seconds. The homogenate is filtered through Whatman No. 1 filter paper on a Coors No. 3 Büchner funnel with slight suction. Filtration is normally quite rapid and when the residue becomes dry, pressure is applied with the bottom of a beaker to ensure maximum recovery of solvent. The filtrate is transferred to a 500-ml graduated cylinder, and, after allowing a few minutes for complete separation and clarification, the volume of the chloroform layer (at least 150 ml) is recorded and the alcoholic layer removed by aspiration. A small volume of the chloroform layer is also removed to ensure complete removal of the top layer. The chloroform layer contains the purified lipid.

For quantitative lipid extraction the lipid withheld in the tissue residue is recovered by blending the residue and filter paper with 100 ml chloroform. The mixture is filtered through the original Büchner funnel and the blendor jar and residue are rinsed with a total of 50 ml chloroform. This filtrate is mixed with the original filtrate prior to removal of the alcoholic layer.

Adaptation to Other Materials

The above procedure can be applied directly to any 100-g sample containing 80 g water. Many alterations of the procedure are permissible but it is imperative that the volumes of chloroform, methanol, and water, before and after dilution, be kept in the proportions 1:2:0.8 and 2:2:1.8, respectively. These ratios represent the total volumes present in the ternary systems, including the water present in the sample. Thus, in the extraction of materials that do not contain 80% water, either the size of the samples can be adjusted so that they contain 80 g water, or 100-g samples can be used and the volumes of chloroform and methanol changed to give the correct proportions. In cases where the moisture content is much less than 80% (e.g. fish meal), it is necessary to add distilled water. When material containing a large amount of lipid is used, or where the supply of material is limited, the size of sample can be reduced and the above solvent quantities scaled down to meet requirements.

Determination of Lipid Content

A portion of the lipid extract containing 100–200 mg lipid is evaporated to dryness in a tared flask and the weight of the lipid residue determined. Evaporation, facilitated by a stream of nitrogen, is carried out in a water bath at 40–50° C and the residue is dried over phosphoric anhydride in a vacuum desiccator. After weighing, a small volume of chloroform is added to each flask to detect the presence of non-lipid material (insoluble). If non-lipids are present, the chloroform is carefully decanted and the flask rinsed three times with chloroform. The dry weight of the residue is determined and subtracted from the initial weight. The lipid content of the sample is calculated as follows:

$$\text{Total lipid} = \frac{\text{weight of lipid in aliquot} \times \text{volume of chloroform layer}}{\text{volume of aliquot}}.$$

Experimental

Optimum Conditions for Lipid Extraction

The first objective was to find which of various mixtures of chloroform and methanol would yield quantitative extraction. The solvents were mixed in

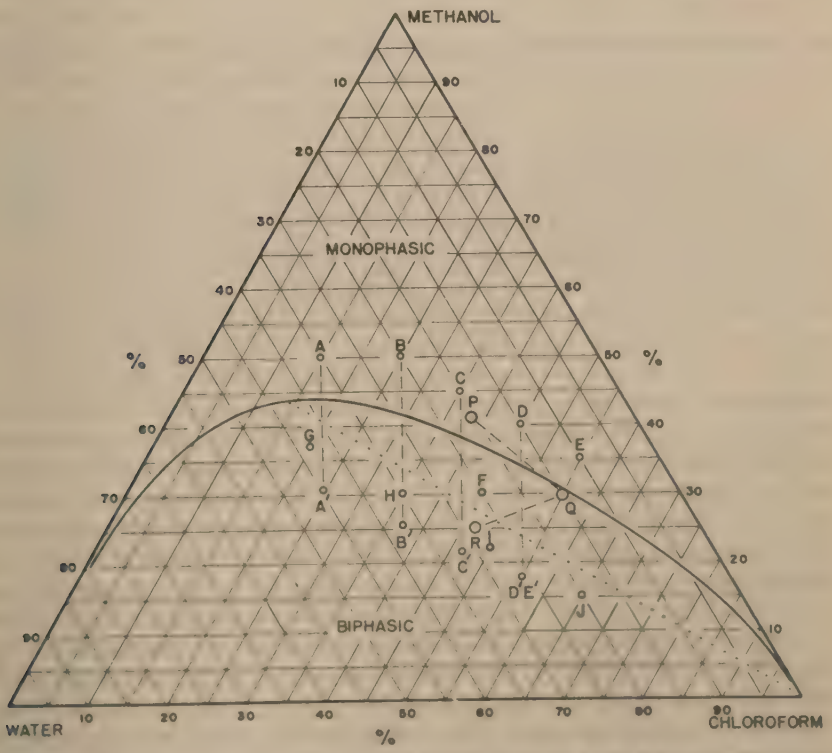

FIG. 1. Chloroform–methanol–water phase diagram, % (w/w) at 20° C (5). ..... "maximum chloroform tie-line" estimated from data for 0° C (6). - - - dilutions.

such proportions that, with the water of the samples, ternary systems were formed having compositions as marked on the phase diagram (Fig. 1, points A–J). Samples of minced cod muscle (100 g each) were homogenized with the extractants (Table I) and filtered. To the monophasic extracts, sufficient water

TABLE I

Lipid extracted from 100 g cod muscle by ternary mixtures of chloroform–methanol–water

Initial extraction mixture				Dilution solvents			
Ref. pt. in phase diagram (Fig. 1)	Chloroform, ml	Methanol, ml	Water,* ml	Chloroform, ml	Water, ml	Total volume, ml	Lipid in chloroform layer, g
A	23	144	80	40	87	374	0.31
B	54	202	80	104	154	594	0.56
C	94	228	80	166	184	752	0.63
D	162	270	80	285	228	1025	0.64
E	296	355	80	290	325	1346	0.62
F	97	121	80	†	†	298	0.56
G	50	174	160	†	†	384	0.32
H	54	87	80	†	†	221	0.32
I	96	80	80	†	†	256	0.40
J	175	76	80	†	†	331	0.41
P	100	200	80	100	100	580	0.70

*Including 80 ml from tissue.
†No additional solvents required to render system biphasic.

and chloroform were added to render the systems biphasic. The final compositions of these ternary mixtures are given by the points A′, B′, C′, and D′E′ in Fig. 1. The lipid contents of all chloroform layers were determined as above.

The results (Table I) confirmed the initial hypothesis in that more lipid was extracted by mixtures of the monophasic area of the diagram than by those of the biphasic area. Mixtures C, D, and E gave the highest values; however, the volumes of chloroform required for D and E were large and inconvenient. Therefore, the area around point C was considered most favorable.

A tie-line has been drawn in Fig. 1 which for the purposes of this paper has been called "the maximum chloroform tie-line". This tie-line was particularly significant in this study since the lower layers of ternary systems having compositions on or below it are practically 100% chloroform (6). Systems having compositions above this line have chloroform layers contaminated with methanol and water. This explains why the chloroform layer of the system represented by point F in Fig. 1 was the only one found to contain non-lipid.

Point P in the monophasic area of Fig. 1 was chosen as a convenient starting point for further investigation since the composition was equivalent with 100 ml chloroform, 200 ml methanol, and 80 ml water. The composition after adding another 100 ml chloroform is represented by point Q in the biphasic area of the diagram. Point R, which is below the maximum chloroform tie-line, is obtained by subsequent dilution with 100 ml water. This sequence was followed with cod muscle. The tissue (100 g) was homogenized with 100 ml chloroform and 200 ml methanol, filtered, and the residue rinsed with 100 ml chloroform in three portions. The pooled filtrates were mixed with 100 ml distilled water and the mixture was allowed to separate in a 500-ml graduated cylinder. Separation of the layers was rather slow but satisfactory. The

chloroform layer contained 0.70 g lipid, the highest yield obtained for this lot of cod muscle (Table I).

Further experimentation showed that the same result was obtained when dilution with chloroform and water preceded filtration. The chloroform had to be added before the water and each addition followed by blending. This resulted in a rapidity of separation of the layers and a yield of lipid which could not be obtained by adding the solvents in a different order.

The extraction of frozen samples did not significantly lower the final temperature of the extract; consequently it was unnecessary to thaw samples prior to extraction.

Substitution of glass fiber filter paper for Whatman No. 1 filter paper showed that with cod flesh there was no significant loss of lipid with the latter due to adsorption. Sintered glass filters also had no advantage over the regular filter except perhaps with materials such as fish meal.

Non-Lipid in Chloroform Layer

It was shown by three methods: (*a*) the determination of chloroform-insoluble material, (*b*) purification according to Folch *et al.* (2), and (*c*) purification according to Shorland *et al.* (7), that the lipid extracted by the above procedure contained no significant amounts of non-lipid material. The amounts of impurities, if present at all, were below the sensitivity limit of each of these methods.

Lipid in Methanol–Water Layer

The methanol–water layer was quantitatively removed and evaporated to less than 50 ml by distillation under reduced pressure at 40–50° C. The concentrate was quantitatively transferred to a separatory funnel and extracted four times with an equal volume of ethyl ether. Any emulsions were broken by centrifugation. The weight of lipid recovered from the pooled extracts was 8 mg. This loss, being only about 1% of the total lipid, was considered insignificant in most applications.

Lipid Remaining in Tissue Residue

Several procedures were employed to recover any lipid remaining in the extracted tissue. The most effective procedure involved re-blending the tissue residue and filter paper with 100 ml chloroform, followed by filtration and rinsing of the blendor jar and residue with a total of 50 ml chloroform. The recovered lipid weighed 40 mg, approximately 6% of the total extracted lipid. Re-extraction of the washed residue with chloroform–methanol–water did not yield any further amount of lipid. Thus, the initial extraction isolated approximately 94% of the extractable lipid.

The washed residue was further treated with 200 ml 6 *N* hydrochloric acid at 100° C for 90 minutes to establish whether there was lipid remaining which was bound in such a way that chloroform–methanol would not remove it. The liberated "lipids" were extracted from the digest according to A.O.A.C. specifications (8). The yield was 47 mg (Table II). At least part of this material may have originated from inositides (see (9)), the protein complexes

of which have been found to be resistant to chloroform–methanol extraction (2). Furthermore, the drastic acid treatment may have produced some fat-like material from non-lipid constituents of the muscle.

TABLE II

Grams of lipid extracted from 100 g cod muscle by four methods

Present method	Method of		
	Dambergs (1)	A.O.A.C. (9)	Folch *et al.* (3)
Sample I			
0.65	—	0.63	—
0.67	—	0.62	—
0.66	—	—	—
0.68	—	—	—
Sample II			
0.76	0.67	—	0.78
0.76	0.69	—	0.77
0.05*	0.07*	—	0.02*

*Lipid isolated from residue after digestion with hydrochloric acid.

Comparison with Existing Methods

Samples of well-mixed minced cod muscle (100 g each) were extracted using the described method and the methods of Dambergs (1), Folch *et al.* (3), and A.O.A.C. (8). The results are shown in Table II. The average lipid content of sample I as determined by the present method was 0.666% (standard deviation, 0.013), which was higher than the values obtained using the A.O.A.C. method (8). For sample II, which had a higher lipid content than sample I, the method of Folch *et al.* (3) and the present method gave yields which were not significantly different; however, the extract of the former method usually contained small amounts of non-lipid material. The values obtained using the method of Dambergs (1) were somewhat lower than those of the other methods.

The flesh of several species of fish (100-g samples) with different fat contents, and two lots of fish meal (10-g samples) were analyzed by the described method

TABLE III

Grams of lipid extracted from various samples using the present method and the method of Dambergs (1)

Sample*	Present method	Method of Dambergs (1)
Halibut fillets	0.75†	0.74
Cod fillets (pre-rigor)	0.76	0.68
Cod fillets (5 yr at −10° F)	0.56†	0.53
Cod fillets (18 mo at +10° F)	0.50†	0.49
Swordfish steaks	7.85†	6.51
Rosefish fillets	1.88	1.68
Plaice fillets	0.66	0.68
Fish meal (A)	0.34	0.30
Fish meal (B)	0.54	0.48

*All samples except the fish meals were 100 g; of the meals 10 g was used per extraction.
†Tissue residue was not washed.

and that of Dambergs (1). The results in Table III show that the new method for the most part extracted more lipid than the method of Dambergs (1).

Discussion

When the proper proportions of chloroform and/or water are added to a miscible chloroform–methanol–water mixture, the system separates into two layers. The proportions may be chosen in such a manner that the lower layer will be practically 100% chloroform and the upper layer nearly all methanol–water. This concept was applied to the extraction and purification of tissue lipids. It was found to be very effective; the miscible solvent mixture was an efficient lipid extractant which upon the addition of chloroform and water separated into two layers and in so doing separated the lipids from the non-lipids. The method which was developed is simpler and less time-consuming than procedures hitherto reported and is well adapted to routine lipid analyses, and also to lipid composition studies since only mild treatment of the samples is involved. For practical purposes, total lipid extraction was complete; slightly more fatty material could be isolated only after hydrochloric acid digestion. The separation of lipids and non-lipids was nearly quantitative. The results of lipid determinations on a sample of cod muscle were reproducible to within $\pm 2\%$ (standard deviation).

In some applications it may be unnecessary to wash the tissue residue since the washings contained only 6% of the total lipid and the lipid composition of the washings, as shown by free fatty acid and phospholipid determinations, did not appear to differ from that of the original extract.

Some chloroform is evaporated during filtration of the homogenate but this is not serious provided the decrease in volume is not too great.

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Age Determination in Odontocete Whales from Dentinal Growth Layers

By

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Introduction.

Growth layers in the dentine and in some species in the cement of the teeth have been used successfully in recent years to determine the age of both Phocid and Otariid seals. An absolute age scale has been obtained either from animals marked as young (Scheffer 1950), or by collecting teeth throughout the year and studying the seasonal sequence of deposition of layered material (Laws 1952, 1953a; McLaren 1958).

According to Nishiwaki and Yagi (1953) Odontocete whales have long been known to possess a layered or striped structure in the dentine of the teeth. These authors studied the teeth of a dolphin *Prodelphinus caeruleo-albus* (now *Stenella c. alba)* (Meyen) which shows regular layering in the dentine. They attempted to study the rate of deposition of dentine material by injecting lead salts into captive animals, and after death measuring the amount of lead deposited in the teeth following suitable fixation and sectioning. Unfortunately their captive animals did not survive long enough for significant deposition of lead-stained dentine to occur. Lacking known age material these authors were not able to determine the absolute frequency of appearance of the layers or stripes.

Yablokov (1958) figured layered growth in sections of the teeth of the white whale *Delphinapterus* and sperm whale *Physeter*. In these species layering is seen both in the dentine and the thick cement. Lankester (1867) also figured layering in the cement of the teeth of *Ziphius*. However, neither of these workers commented on the possible significance of the layers. Omura, Fujino and Kimura (1955) noted layered growth in the cement of the teeth of *Berardius bairdi* from which they thought it might be possible to determine age.

Nishiwaki, Ohsumi and Hibiya (1958) studied the sequence of layered growth in the mandibular and hidden maxillary teeth of the sperm whale: the maxillary teeth do not wear at the tips as do the large mandibular teeth and are thus more useful for age determination. These authors compared the observed number of dentine layers with growth in length and with the number of corpora albicantia in the females. They found up to 40 or 50 growth layers in the largest animals. Female sexual maturity occurred when about 9 growth layers had been laid down, male sexual maturity at 9 or 10 growth layers. From the number of growth layers in the teeth of the smallest animals collected, and by analogy with the fin whale, Nishiwaki, Hibiya and Ohsumi concluded that two growth layers are laid down a year in the sperm whale. The ear plug of the fin whale has been shown to grow by two complete layers, each consisting of a light and a dark zone, in one year (Laws and Purves 1957, Nishiwaki 1957).

The chief obstacle to a reliable age-scale in whales is the absence of known-age animals. With the large whales one may hope that in the future it will be possible to mark calves in larger numbers than hitherto. With the small porpoises and dolphins the recent development of colonies of captive animals, some born and raised wholly in captivity, promises to provide valuable material. In April 1958 the writer visited Marine Studios, Florida, the oldest marine aquarium in the United States with

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such a porpoise colony, and was fortunate in obtaining teeth from two specimens of the bottlenose dolphin or porpoise, *Tursiops truncatus* (Montagu), which had been reared wholly in captivity, as well as from two wild-caught animals which had lived subsequently in the aquaria. This material throws light on the aging of Delphinids. The writer has also studied teeth and reproductive material from a large number of wild pilot whales *Globicephala melaena* Traill and of white whales *Delphinapterus leucas* (Pallas) and a preliminary discussion of the results of aging these two species follows the treatment of *Tursiops*.

Material.

Dates of birth or capture and of death, together with lengths and weights at death, are given in Table I for the four *Tursiops* obtained from Marine Studios. The wild-caught «Mona» gave birth in captivity on February 26, 1947; that is 10 months after capture. It was believed that this calf was her first. The daughter, «Spray», herself first gave birth at seven years of age, after a twelve months pregnancy (Tavolga and Essapian 1957). Thus «Mona» had probably reached sexual maturity and had been impregnated shortly before capture. If the maturation of «Spray» was normal for the species, that is neither accelerated nor retarded in captivity, then «Mona» was aged about 6 years at capture and 15½ years at death, 9½ years later.

Table I. Vital statistics of *Tursiops truncatus* from Marine Studios.

No.	Name	Sex	Dates of: Birth	Capture	Death	Known age	Length at death	Weight at death
1.	Rollie	Male	March 6, 1955	—	Sept. 1, 1955	6 mo.	4 ft. 5 in. (135 cm.)	56 lb. (25 kg.)
2.	Maggie	Female	Feb. 23, 1953	—	July 12, 1955	2 yrs. and 4½ mo.	7 ft. 1 in. (216 cm.)	215 lb. (97.5 kg.)
3.	—	Male	Unknown	Dec. 18, 1957	April 16, 1958	?	7 ft. 8 in. (234 cm.)	400 lb. (181 kg.)
4.	Mona	Female	Unknown	April 27, 1946	Aug. 28, 1955	? (est. at 15½ yrs.)	8 ft. 2 in.) (249 cm.)	305 lb. (138 kg.)

Macroscopic appearance of the teeth.

The teeth of *Tursiops* are set close together in a deep groove of the maxillary bone. The crowns are curved inward. In the 6 month animal their length is about 17 mm. and the root extends to within 4 mm. of the base of the groove. At 2½ years the teeth are set more tightly in the bone, the roots curving backward along the groove. There is little wear of the crowns and these project further from the gum. At a supposed age of 15½ years the teeth are larger and project considerably further from the maxillary bone. The tips are worn to a slight degree, the wear being greatest on the most anterior teeth. The teeth are surrounded by stellate bony outgrowths of the maxillary which hold them more tightly; in the younger animals they are surrounded by bands of fibrocartilage but there is no ossification. In the 2½ years animal the pulp cavity of the tooth is wide, in diameter about 50 % of the diameter of the root, but at 15½ years the pulp cavity has shrunk to only 5 % of root diameter.

Each jaw contained about 18 teeth of the maxillary series (the full number in each jaw in *Tursiops* is about 22). The teeth were subequal in size from tooth 6 onwards while numbers 1 and 2 were very small and numbers 3 to 5 considerably larger but not of full size.

Preparation of tooth sections.

About the 15th to 18th teeth from the anterior end of the maxillary series were removed for sectioning. They were cut into longitudinal halves from which thin longitudinal sections were cut. A fine-toothed circular sawblade was used mounted on a grinding head and run by a ¼ HP motor. To cut a section a half-tooth was glued to a wooden block with plastic wood. The section was then polished on a fine grindstone until translucent when held up to the light. Sections were also decalcified in 70 % alcohol with 5 % HCl and stained in Delafield's haematoxylin. The slower decalcification of whole teeth, followed by wax embedding and sectioning was not employed but would have given more even sections.

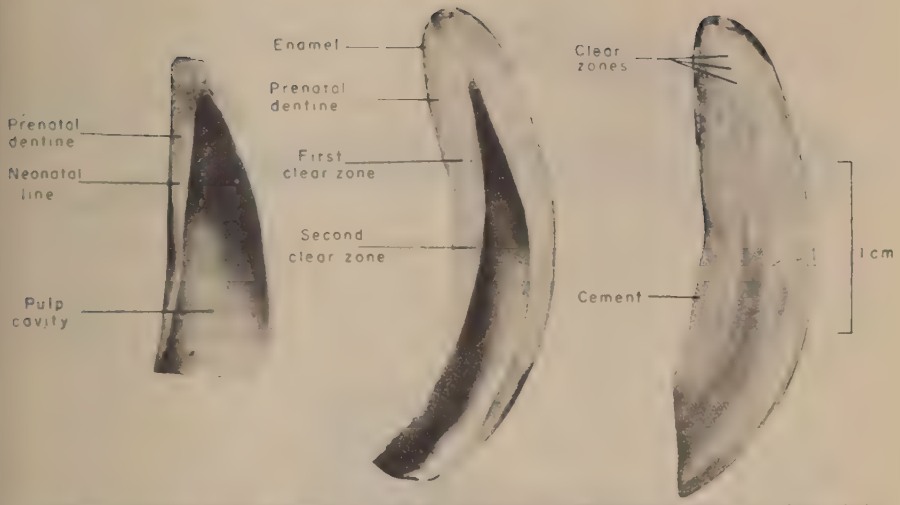

Figure 1. Longitudinal half-teeth of *Tursiops truncatus*, the flat faces of which have been photographed by reflected light. Left, the 6-month-old male; centre, the $2\frac{1}{2}$-year-old female; right, the old female possibly 17 years old. The root of the tooth of the old female is broken off near the base.

Results.

Structure of the Delphinid tooth.

The structure of the teeth is shown in Figures 1 to 3. Figure 1 shows the flat faces of half-teeth of three of the animals, as viewed by reflected light.

Opaque dentine here appears light in colour since it reflects light, while translucent dentine appears dark since it absorbs and scatters light. The reverse appearance is shown by thin sections photographed with transmitted light (Figure 2 upper and Figure 3). Figure 2, lower, shows a decalcified, stained section. The intensity in staining is seen to follow the optical density of a thin section, that is, haematoxylin selectively stains opaque dentine. More detail is seen after staining.

In a Delphinid such as *Tursiops* the crown of the tooth is well-formed at birth, prenatal dentine being capped by a thin layer of enamel. The root, consisting of a very thin layer of dentine at birth, elongates and thickens throughout life by deposition of successive cone-shaped increments of dentine.

Nishiwaki and Yagi's excellent description and figures of the structure of the teeth of *Stenella* could apply as well to *Tursiops*. These authors used both thin, ground sections and decalcified, frozen sections stained with haematoxylin and eosin. The calcified part of the prenatal dentine in a full-term dolphin foetus was uniform (unstriped) and fairly dense to light, and stained well with haematoxylin. The neonatal line, laid down shortly after birth, was clear and unstainable. Following the neonatal line came alternating zones of dense, heavily-staining dentine and clear, poorly-staining dentine, repeated at regular and gradually decreasing intervals until the pulp cavity was reached.

Schour and Massler (1949) studying the teeth of the rat came to the «empirical but reliable conclusion» that haematoxylin selectively stains well-calcified tooth material, leaving poorly calcified to its counterstain. The staining of such tissues after decalcification is explained by Carleton and Drury (1957, p. 182) who state that haematoxylin stains the ossein matrix of the bone. I have obtained consistent results after staining decalcified dentine with Delafield's haematoxylin or Heidenhain's iron haematoxylin, though undecalcified thin sections gave variable results. Dentine believed to be well-calcified from its optical density stained deeply with haematoxylin. Moreover the neonatal line which is formed during the period of adaption and consequent disturbed nutrition immediately following birth remains clear and unstained. Nishiwaki and Yagi point out that, where the neonatal line reaches the surface of the tooth halfway down the root, the contour of the surface is marked with a

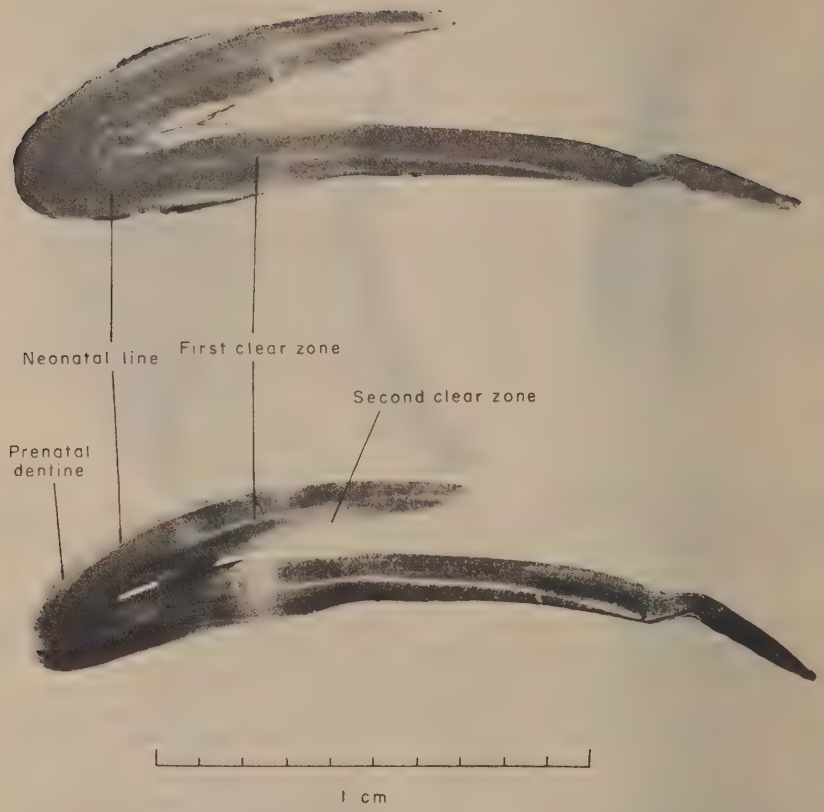

Figure 2. Longitudinal thin sections of teeth of the 2½-year-old female *Tursiops*, which died in July. Above, thin ground section photographed by transmitted light; below, ground, decalcified section stained with Delafield's haematoxylin. The neonatal line and first stripe are more distinct than the second stripe. The position of the second stripe shows that it was laid down in the early spring.

distinct depression suggesting poor growth (hypoplasia) of dentine at this time. It can scarcely be doubted, therefore, that poor staining indicates poor calcification.

Terminology.

The term «layer» is used for a complete increment, and «zone» for one of its subdivisions.

Growth layers.

The tooth of the 6-month old «Rollie» (Figure 1) shows optical differences between prenatal and postnatal dentine, with a clear neonatal line intervening. The postnatal dentine is generally opaque but shows a clear zone after the neonatal line. On staining, the prenatal dentine is uniformly dark, the neonatal line clear, and the postnatal dentine shows the same variable density as when unstained. The thickness of postnatal dentine is 375 microns as seen in a median longitudinal section (it will of course be wider if the section is tangential). Since the age at death was 185 days, the average daily rate of deposition of dentine was about 2 microns.

The teeth of the 2½ year old «Maggie» are of

key importance. They show two zones of clear, poorly staining dentine in addition to the neonatal line (Figures 1 and 2). One such zone is therefore laid down annually. The first clear zone is narrower and considerably more distinct than the second. The total width of postnatal dentine is about 1550 microns; starting from a clear zone each year this can be divided into 675 microns for the first year and 625 for the second, with 250 microns remaining. Full growth in the third year would presumably be about 600 microns. It can therefore be calculated that the period of growth in the third year was about $\frac{250}{600} \times 365$ days, or 152 days, or 5 months. Since «Maggie» died on July 12, deposition of the second clear zone began about mid-February. The first clear zone is about 50 microns wide, and if one may assume that the rate of deposition of this zone does not differ from the opaque dentine, then it was formed for about one month. The second clear zone, as we have noted, ends indistinctly.

The wild-caught male, held 6 months in captivity and sacrificed in mid-April, shows ten growth layers (Figure 3). The pulp cavity is still wide, showing that addition of several more layers was possible. Total width of the growth layers is about 3 mm., growth declining from about 650 microns in the first layer to about 125 in the last. A stained section shows in each layer an abrupt transition from the clear, poorly-staining zone to the opaque,

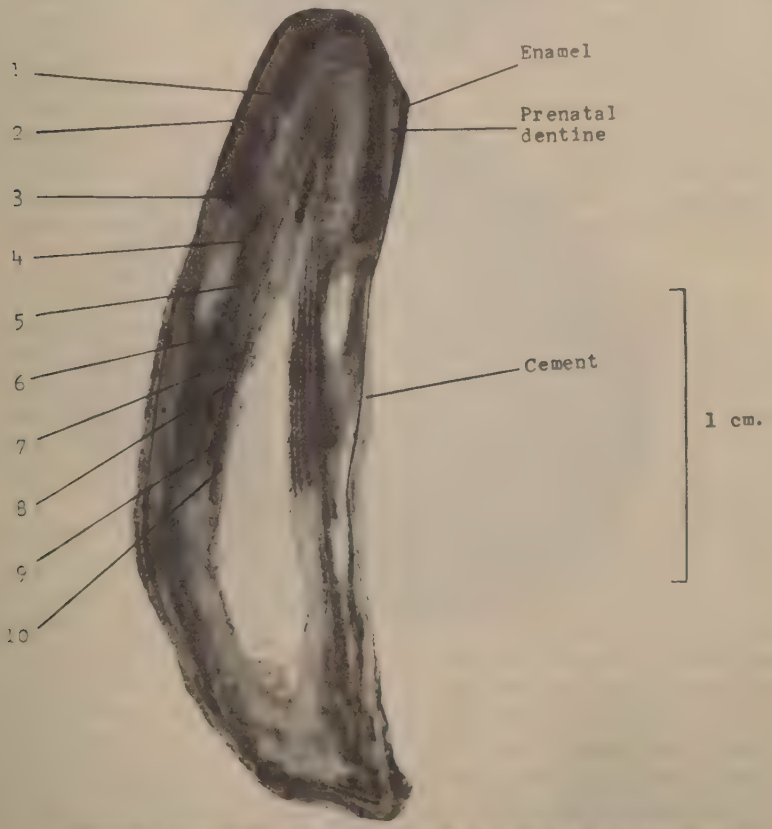

Figure 3 Longitudinal thin ground section of a tooth of the wild-caught male *Tursiops* which was killed in April. About ten growth layers can be counted in the dentine.

deeply staining zone, after which material of intermediate density passes gradually to the next clear zone. This appearance agrees with the optical properties.

The last growth layer having a thickness of only 125 microns, the exact stage of deposition at time of death is hard to determine. However it appears that a clear zone adjoins the pulp cavity and is more or less fully formed.

Teeth of the old female «Mona» show about 9 layers filling the greater part of the dentine. There follow some 8 further layers confined to the central axis and each occupying a small crescentic space. These were only detected in an accurately bisected tooth. In other words the pulp cavity was almost completely filled, being open only towards the base of the root, and allowed only a very small amount of layered deposition after nine years. The total thickness of the dentine on each side is about 1250 microns and the first layer measures 450 microns, a lower rate of deposition than in the 10 year old male. In stained section the teeth of «Mona» again show alternating opaque and clearer zones, with considerably more detail visible than in unstained sections. The age of «Mona» as estimated from growth layers was 17 $\pm$ 2 years, which is in agreement with the 15½ years calculated from the reproductive history.

Discussion.

The foregoing description of the growth-layers in *Tursiops* is identical with that described and figured by Nishiwaki and Yagi for *Stenella,* and one can scarcely doubt that the periodicity of the growth layers is the same. Nishiwaki and Yagi, it is true, describe the «stripe» as made up of opaque dentine, while in our animals the clear zone is the thinner of the two and would logically be called the «stripe». This discrepancy seems to result from the Japanese workers using mostly stained sections, in which darkly staining dentine may make up quite a thin part of each growth-layer. But in unstained ground sections opaque or optically dense dentine seems to be wider in their sections as well as ours. In order to avoid confusion the term «stripe» has not been used in the present paper.

Captive *Tursiops* at Marine Studios mate and give birth between February and May, gestation lasting one year (Tavolga and Essapian 1957). As mentioned above, the second clear zone of the 2½ year old «Maggie» began formation about February, and the last clear zone in the 9-year old male was probably complete in April. When I asked whether there were any annual changes in feeding of captive porpoises, Mr. F. G. Wood replied (letter of December 18, 1958): «I think I can say that, generally speaking, the porpoises tend to go off their food during the early spring months. The season coincides more or less with their mating period». It is not yet clear whether this behaviour is limited to sexually mature animals or whether it occurs, as a result of the social-sexual excitement, in the colony as a whole.

The first clear zone of the 2½ year old animal coincides with a depression on the surface of the tooth well along the root (Figure 1). This character was seen in the neonatal line where it was considered by Nishiwaki and Yagi to show hypoplasia. The lack of staining of the clear zones suggests that they are hypocalcified. It would be reasonable to assume that hypocalcified material is laid down during a period of suboptimal nutrition. This possibly occurs during the breeding period. An alternative or perhaps additional explanation might be that an internal, humoral rhythm controls tooth calcification, and acts on immatures as well as adults. In the ringed seal (McLaren 1958) a period of starvation occurs in the spring when the animals remain on the ice moulting the hair, and during this time the canine teeth lay down clear or vacuolated dentine. At other times of year when good feeding takes place, opaque dentine is laid down.

Other species of Delphinids.

I have examined teeth from some 600 pilot whales or blackfish *Globicephala melaena* (Traill) taken in Newfoundland, and have also studied the reproductive biology. The teeth show much the same structure and sequence of dentinal growth-layers as *Tursiops.* However they are shorter and may be examined after transverse sectioning, a simpler technique than cutting longitudinal sections. A tooth section of an immature animal is shown in Figure 4, upper.

Growth layers are regular enough in the majority of animals to allow determination of age to within one or two layers. After some 12 layers have been deposited the majority of teeth become filled to the centre with dentine but a few animals show up to 21 growth layers. Sampling was limited to a 3½-month period between mid-July and late October when the pilot whales concentrate in coastal waters and feed heavily on squid. For the rest of the year, and in some rare summers, the whales remain in the open sea (Sergeant and Fisher 1957). Birth takes place in summer and mating probably in the spring. From a study of the wide growth layers of young immature animals it was deduced that opaque, heavily-staining dentine is normally

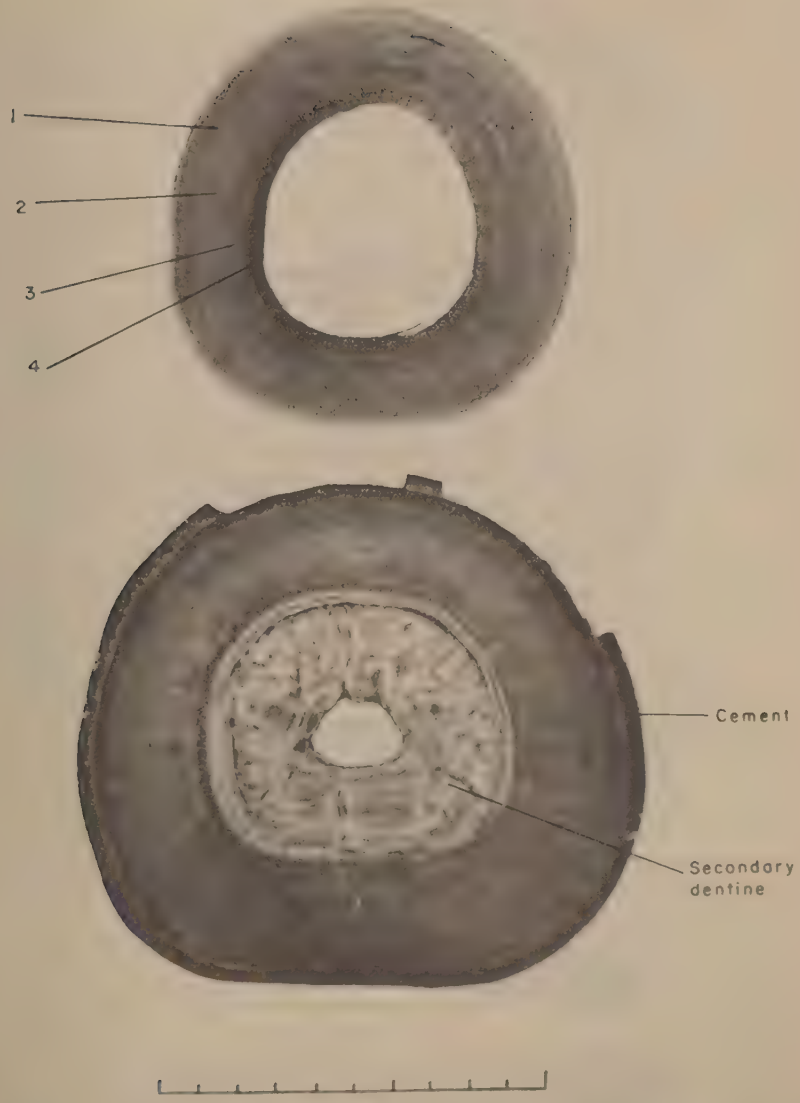

Figure 4. Transverse thin ground sections of teeth of pilot whales. Above *Globicephala melaena*, length 10 ft. 11 in. (333 cm.) from Newfoundland on August 23, 1954. Age estimated at 4 years. Below *Globicephala macrorhyncha*, animal 10 to 11 ft. in length from St. Augustine, Florida, in March, 1958. The subtropical species has a larger tooth with thicker cement, secondary dentine nearly filling the pulp cavity, and more frequent dentine layers than the northern species.

laid down in summer. The clear dentinal zones are rarely laid down at this time. The mean age at sexual maturity of females, on the assumption that one layer is laid down a year, is 6 years; this is the same as the known age at sexual maturity of the single female *Tursiops* raised from birth to maturity to date (Tavolga and Essapian, loc. cit.). A full report on the tooth structure and age characteristics of this population of *Globicephala melaena* is in preparation.

Teeth were examined from a few specimens of several other northern Delphinids taken on the Canadian east coast from New Brunswick to Newfoundland and Labrador. These were; killer whale *Orcinus orca* (one specimen), the dolphins *Delphinus delphis* (1) and *Lagenorhynchus acutus* (1), and common porpoise *Phocaena phocoena* (2). All these showed the same type of growth pattern as the teeth of *G. melaena* or *Tursiops*. The porpoise teeth showed in addition that the spade-shaped crown, typical of the group, is composed of thick prenatal dentine and of enamel. It would seem that all these species are potentially ageable using tooth sections.

I also obtained from Marine Studios teeth from two pilot whales stranded in northern Florida. One of the skulls was identified as *Globicephala macrorhyncha* (Gray) by comparison with the figures of Fraser (1950). The teeth of this species were found to be considerably larger than those of *G. melaena* of the same body size (Figure 4). In section they show much thicker dentine, with early deposition of secondary dentine in the specimen figured, and thicker cement. Growth layers appear to be more frequent than in *G. melaena* of the same length. Nevertheless the length at birth is the same as for *G. melaena,* namely about 5½ ft. (170 cm.), and the adult males reach the same maximal length of about 20 ft. (610 cm.) (Gunter 1946, Kritzler 1952, and Sergeant and Fisher 1953). The wide difference in tooth structure between these closely related species is noteworthy.

The cement of all Delphinid teeth examined was found to be relatively thin but showed growth layers which in some instances could be counted under high magnification.

As mentioned above, the cement of the teeth of white whales is extremely thick and I have found that its growth layers can be counted as easily as those of the dentine. (Figure 5). Several hundred teeth were examined from white whales taken in summer in the Canadian arctic (Churchill in Hudson Bay and the Mackenzie River delta). The material was restricted not only in season but also in being taken from a selective kill consisting mainly of larger animals. As a result I have not yet been able to make a certain estimate of the number of growth layers laid down annually. In the Hudson Bay population the females mature sexually at a length of about 9 ft. (275 cm.) and an age represented by 12 growth layers; the males mature sexually over lengths of 9 to 10½ ft. (275 to 320 cm.) and ages represented by 12 to 18 growth layers. The change in body colour from grey to white also takes place with fair constancy in both sexes when 13 to 18 growth layers have been laid down. The maximum number of growth layers shown in the oldest animals is about 50, 35 layers being commonly attained. These values are similar to those obtained by Nishiwaki, Ohsumi and Hibiya for the sperm whale. It therefore seems possible that two complete growth layers are laid down annually in the white whale which would give an age at maturity of 6 years for the female and 6 to 9 years for the male. Dorofeev and Klumov (1936) basing their results on an analysis of body length frequencies concluded that sexual maturity occurred at 3 years in the female and 4 years in the male white whale. Recent work on seals (Laws 1953b, 1958) has shown that individual variation in length in seals obscures all but the first age group and that consequently length frequencies are an unreliable guide to age. The ages at sexual maturity in seals, as determined from the teeth, have usually proved greater than had previously been estimated from length frequencies. It seems likely that the same will be found to hold true for Odontocete whales.

Two young white whales have recently been captured and held in a Californian marine aquarium (Marine Enterprises Inc.) and other aquaria are making efforts to obtain specimens. It seems likely that within a few years the growth rate of the white whale will be known and it will then be possible to assign an absolute age scale from growth layers in the teeth.

Summary.

A review is given of previous work on age determination from growth layers in the teeth of Odontocete whales.

Teeth from 4 bottlenose dolphin *Tursiops truncatus* showed regular growth layers in the postnatal dentine. Each layer consisted of a wide zone of opaque, deeply-staining material and a narrower zone of clear, lightly-staining material. A 2½ year-old animal reared in captivity showed that one growth layer was laid down each year. In this animal the second clear zone was laid down in early

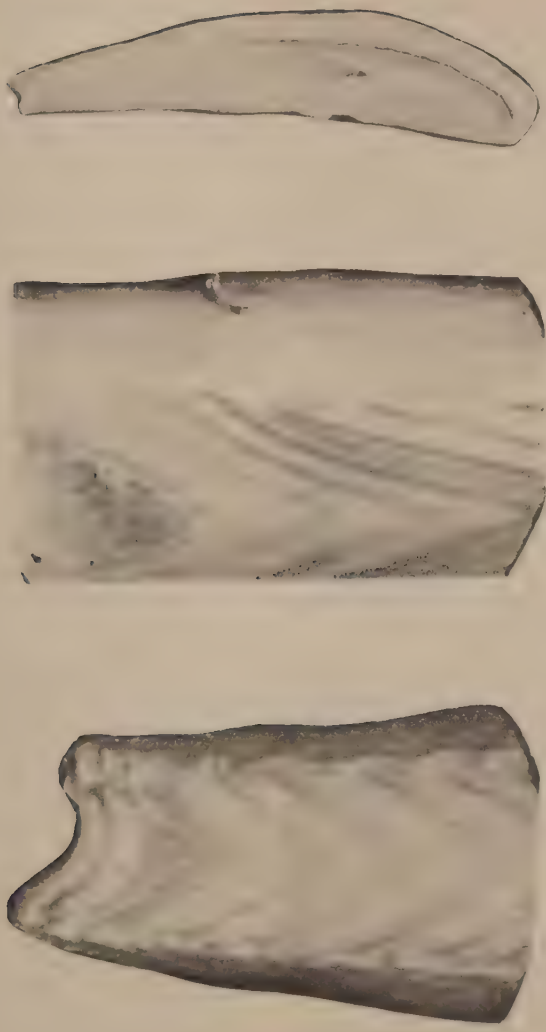

Figure 5. Longitudinally bisected tooth from upper jaw of white whale *Delphinapterus*. The animal was an adult male, length 11½ ft. (350 cm.) from Churchill, Hudson Bay. Upper: whole view. About 30 growth layers can be seen in the dentine. Centre: detail of central portion of tooth showing growth layers in dentine, locally interrupted by deposition of secondary dentine, and in cement. Lower: detail of base of tooth showing the growth layers most recently added to the dentine.

spring. The layering probably indicates variations in calcification of the dentine. The mechanism of the layering remains obscure but it is possible that changes in feeding play a part.

Other Delphinids, particularly pilot whales *Globicephala melaena*, were studied and demonstrated a layered structure of the dentine similar to that found in *Tursiops*. Teeth of white whales *Delphinapterus* showed readable growth layers in both dentine and cement. The maximum number of growth layers was about 50, more than twice as many as found in pilot whales and the same as found by other workers in sperm whales. It is therefore believed that white whales, like sperm whales, may lay down two complete growth layers annually in their teeth (but see Appendix II).

Acknowledgements.

Mr. F. G. Wood Jr., Curator of the Marineland Research Laboratory and Mr. B. C. Townsend Jr., Assistant Curator, provided the unique materials and history which made this work possible. Messrs. W. F. Pollock and R. Szavay made the photographs. Mr. Wood, Dr. J. C. Moore of the American Museum of Natural History, Dr. H. D. Fisher, Dr. A. W. Mansfield and Mr. I. A. McLaren of the Arctic Unit criticised the manuscript at various stages of its preparation. I wish to express my gratitude to all these people for so much important help.

Appendix I.

Since submission of the manuscript a further tooth of *Tursiops* has been received from Curator F. G. Wood Jr. at Marine Studios. Statistics are as follows: name — Splash, sex — male. Captured 17th April 1952, died 9th February 1959, i.e. duration of life in captivity 6 years, 10 months. Length at capture 6 ft. (183 cm.) and age believed 1½ to 2 years. Length at death 7 ft. 7 in. (231 cm.) and weight 247 lb. (121 kg.). Sectioned tooth showed 8 growth layers having a total thickness of about 2800 microns, leaving a wide pulp cavity. The sequence ended with a clear zone in process of deposition. The age at capture must have been just over one year. This tooth confirms that the clear zone is laid down in spring.

Appendix II.

I have also re-examined the teeth of *Globicephala melaena*. Using thinner sections (15 microns) and a magnification of about × 250 under the compound microscope, I have been able to count or estimate the cemental layers in about half the animals. Taking the largest animals of both sexes I found that 30 to 35 growth layers were not rare, 50 layers the maximum. This is the same number as determined for a comparable sample of white whales, not half as deduced from the incomplete dentinal layers.

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STUDIES ON STORED OYSTERS (CRASSOSTREA VIRGINICA)

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Abstract

Survival, fatness and flavour were followed, December to May, in oysters left on beds and in oysters held in air storage at temperatures simulating those of a commercial cold storage, of a cold basement and of a warm basement.

In all respects oysters from beds were best and showed few changes. Survival, fatness and flavour of air-stored oysters decreased remarkably and were successively poorer at higher temperatures.

Storage flavour is detectable after 4 or 5 days in air at 60°F. but clears when oysters are placed in water at 32° or warmer.

During air storage drying seems to be important in limiting survival and is hard to control.

During hibernation at seawater temperatures near freezing, and during air storage, inner faces of oyster shells are etched by acid products of anaerobic respiration. This etching is too vague to be used as an index to storage age.

The results suggests that suppliers of oysters should consider modifying methods of handling, particularly winter handling. Good methods of water storage might improve earnings directly by increasing survival rates of stored oysters. Besides, they should reward suppliers indirectly because the superior fatness and flavour of water-stored oysters should encourage consumption.

In eastern Canada, as in most countries, consumers of oysters on the half-shell do not become oyster-minded until the cool weather of autumn and winter is upon them. Our main fishery therefore takes place between mid-September and late November after which most oyster-producing inlets become ice-bound. The freeze-up brings an end to oyster fishing.

Until the recent devastating epidemic oyster disease Buctouche River, N. B., was an exception to this general rule. There it had long been the custom to fish oysters from the deep steep-sloping sides of the river channel throughout the winter. It was common to see fishermen plunging long-handled (20 to 30 feet) rakes through holes in the ice or tossing oysters culled from their rake-heads into tubs

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of sea water resting on hand sleds. The sea water kept the oysters from freezing.

These winter-fished oysters were produced in small quantities but consumers praised them for their fine quality -- especially their flavour. Our other winter consumers have always eaten oysters that were air-stored since the previous October or November. The supply of these was much greater but it too was, and still is limited. It is usually exhausted by March by which time the oysters have been out of water for four months. Sometimes the supply lasts longer. In June 1952, according to Dr. R. R. Logie, the late Samuel Andrews, oyster grower and proprietor of the seafood restaurant, "Chez Pauze," Montreal, P. Q., was serving his patrons Shippegan, N. B., oysters that had been fished in December 1951. Apparently they were well received after six months' storage. On August 13 this year I sampled Miscou Harbour, N. B., oysters fished November 17, 1957, and shipped by train to Dr. Logie for storage at Ellerslie, P. E. I. After their eight months out of water they had a storage flavour but were quite edible.

From this it appears that oysters are air-stored, alive in the shell, for longer periods in eastern Canada than anywhere else in the world. This peculiar practice creates a whole complex of problems. They comprehend physical engineering problems in temperature and humidity control of storage houses; economic problems, because costs of storage and market acceptance of stored oysters both vary with period of storage and, finally, biological and biochemical problems which are inevitable in dealing with live animals. Few of these problems are well understood but everyone concerned in the oyster industry knows they are important and should be dealt with.

Needler (1934 & 1941) has offered general advice on storage to oyster producers and storehouse operators. Friedman (1933 & 1934) treated one problem of storage -- the risk of moving frozen oysters and its biological complications. Dugal and Fortier (1941) and Baker et al (1942) studied biochemical changes in our species when stored in the shell and Kuhnelt (1938) studied changes in the European oyster (Ostrea edulis). The work I report here attempted to co-ordinate and use results from all these sources. I am grateful to Dr. A. W. H. Needler, my former director, for his efforts in advancing the study.

STORAGE EFFECTS ON SURVIVAL, FATNESS AND FLAVOUR

Test stock for this experiment was fished from a deep (6 to 8 feet) part of the Cooper Bed in Malpeque Bay, Prince Edward Island, (Needler 1931) in the first week of December 1939 (water temperature about 34°F.) and held in an unheated warehouse of the Department of Fisheries' Experimental Oyster Farm. Warehouse temperatures are believed to have been consistently below 40° but not below 32°. On December 23 a barrel and a half of these, selected for uniformity of

Table 1. Summary of winter observations on oysters taken directly from their native bed and from lots of 170 that were variously stored in air for four months after late-autumn fishing.

Treatment of Oysters and Features Observed	Observation Times: Early Winter	Mid-Winter	Late Winter
t in Water on Native Bed			
ate Observed	7 December 1939	23 Feb. 1940	1 May 1940
umber Dead (recently dead shells taken while fishing sample)	apparently none	apparently none	apparently none
ndex of Condition	114	115	110
mell and Taste	Good (100)	Faint storage smell and taste (90)	Good (100)
Stored at 34°F.			
ate Observed	23 December 1939	8 Feb. 1940	23 April 1940
umber Dead (total to date)	0	1	70
ndex of Condition	126	117	112
mell and Taste	Good (100)	Mild storage smell and taste (75)	Strong storage smell and taste but edible (50)
Stored at 41-60°F.			
ate Observed	23 December 1939	8 Feb. 1940	23 April 1940
umber Dead (total to date)	0	27	104
ndex of Condition	126	112	75
mell and Taste	Good (100)	Smell strong; taste unpleasant; inedible (25)	Smell too offensive for tasting; inedible (0)
Stored at 48-53°F.			
ate Observed	23 December 1939	8 Feb. 1940	23 April 1940
umber Dead (total to date)	0	46	130 (all but 4)
ndex of Condition	126	109	-
mell and Taste	Good (100)	Smell worse than 41-60°F. sample; inedible (0)	Stinking; inedible (0)

size ($3\frac{1}{2}$ to $4\frac{1}{2}$ inches), shape (deep cupped and symmetrical) and shell condition (unbroken edges) was moved to the basement of the nearby Biological Sub-Station. They were carefully placed, one layer deep, in shallow tanks of flowing sea water which varied in temperature from 47° to 50°F. Most of them opened their shells within a few minutes and were pumping.

After two hours of this treatment three lots of 170 were selected. Only oysters which closed their shells quickly when touched were chosen. Each lot was then placed in a covered box with a maximum-minimum thermometer and the boxes were disposed as follows:

1. Just outside the Sub-Station in a large wooden vault lined with rock wool -- temperature thermostatically controlled at 34°F.;

2. In one corner of the basement of the Sub-Station where it was cool and damp -- temperature range 41° to 60°F.;

3. In the sub-basement of the Sub-Station which was cool and damp -- temperature range 48° to 53°F.

At the beginning of the test a sample of 20 oysters was withdrawn, some being taken from each lot. Ten were used to measure index of condition (Medcof & Needler 1941) and ten to check flavour by smelling and tasting by the author and at least one other person including Dr. A. W. H. Needler and sometimes Mr. Claude Hayes. Approximately once every three weeks the boxes were opened, the thermometers were read and reset and the oysters were sprinkled with fresh water to offset drying.

In mid-winter and in late winter the oysters in each box were culled. All dead (gaping) oysters were removed and 20 of the survivors were removed from each lot except at the last culling when there were too few survivors in the third box to supply this number. From each lot of 20, 10 were used for index of condition determination and 10 for smelling and tasting.

As a control, similar observations were made at approximately the same times on samples of oysters taken directly from a marked shoal-water area on the Cooper Bed. The February 23, 1940, sample was fished through the ice. At this season the water temperature varies between 30 and 32°F. The May 1, 1940, sample was fished when ice first cleared from the bed and when water temperature fluctuated about 40°F.

The observations are summarized in Table 1 in which all data are numerical, except certain records of smell and taste. To reduce these to a numerical basis for graphical representation the several ratings were assigned arbitrary values as follows:

Good	= 100
Faint storage smell and taste	= 90
Mild storage smell and taste	= 75
Strong storage smell and taste but edible	= 50
Strong storage smell and unpleasant lingering taste; inedible	= 25
Offensive smell preventing tasting; inedible	= 0

The appropriate numerical rating for each sampling according to this scale is shown in the table in brackets opposite verbal descriptions of smell and taste.

Figure 1 is a graphic representation of the tabulated records.

DRYING DURING AIR-STORAGE

During the experiment just described it appeared that the oysters had dried up considerably in spite of periodic sprinklings. Not only were the shells dry but there was a noticeable reduction in the amount of shell liquor. Besides this, the meats of most oysters in the late-winter sample from the basement lacked turgidity, as if they had lost water and some had gas bubbles in their heart ventricles. The same was true for both the mid-winter and the late-winter samples from the sub-basement.

A simple experiment was performed to explore drying. On October 8, 1943, a sample of 10 adult oysters fished the day previous was scrubbed in fresh water and stood on end to drain. At the same time the shells of ten larger oysters, which had just been opened, were scrubbed and set to drain. After 5 minutes when both lots were still wet but not dripping, they were weighed and laid out singly on the laboratory table. They were reweighed several times in the next 9 days. In this period the laboratory temperature varied from 50 to 70°F. and the relative humidity from 48 to 57%. The weighings are summarized graphically in Figure 2.

These oysters were not observed to open their shells but they may have opened inconspicuously.

DEVELOPMENT AND CLEARANCE OF STORAGE FLAVOUR

From the first experiment it was obvious that storage flavour (smell and taste) develops early during air storage and it also appeared that a similar but faint flavour may be present even in oysters

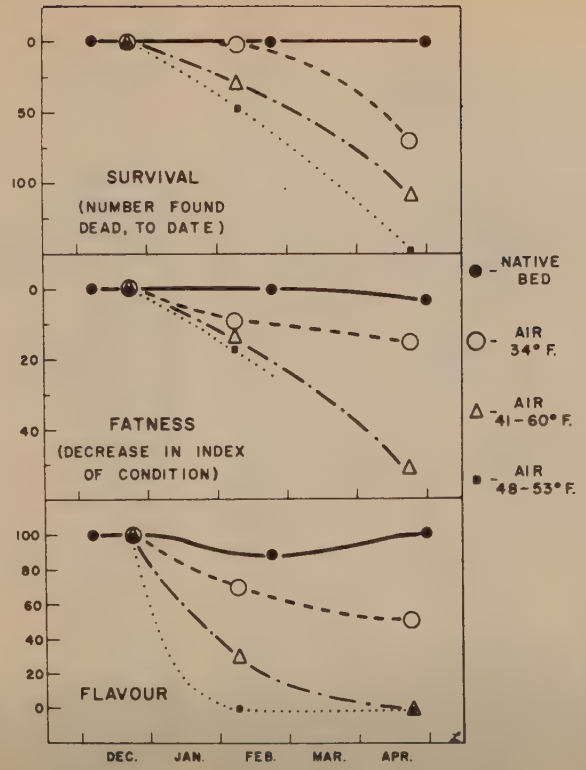

Fig. 1. Survival, fatness and flavour of oysters taken directly from their native bed and from lots of 170 that were variously stored in air for 4 months after late-autumn fishing.

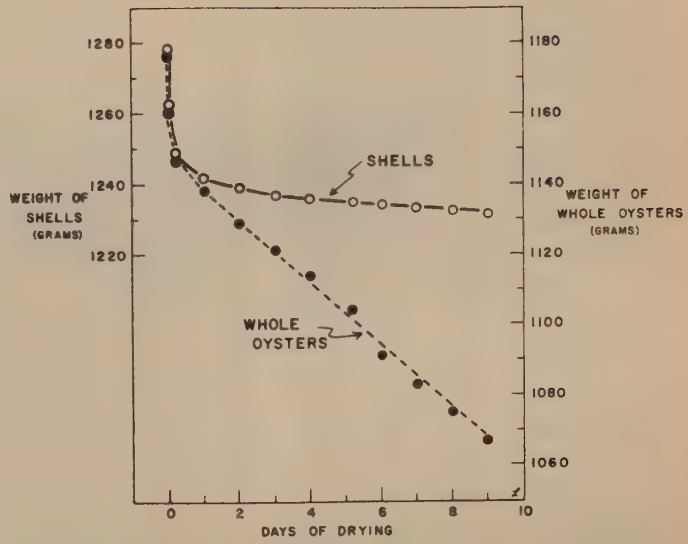

Fig. 2. Weight changes during drying of empty shells and whole oysters exposed on laboratory table.

that are hibernating in water on their native beds when water temperature remains constantly below 32°F. for long periods (Needler 1941).

A controlled experiment was carried out to explore the rate at which these characteristics develop. Oysters were fished in late November 1942 from the Cooper Bed and brought to St. Andrews, N. B. Some were air-stored at 45°F. in the basement of the Biological Station. The rest were stored in a tank of circulating sea water whose temperature varied from 30 to 41°F. From time to time lots of oysters were removed from this cold tank and air-stored for various periods in the basement where the temperature was 60°F. Subsequently some of these lots or parts of them were replaced in the cold tank or in another tank of circulating sea water at 60°F. Samples of these lots were compared for flavour both among themselves and also with samples drawn directly from the cold tank.

In all this handling which sometimes involved shifting back and forth from water to air several times (Table 2, tests 5 and 6) none of the oysters showed adverse effects.

Samples to be tested were brought into the laboratory and laid out on a table for at least one hour to let them all come to approximately room temperature. They were then opened and each sample subdivided into groups of three and labelled with code numbers but not otherwise identified. In any test each taster was presented with two groups and asked to state his preference. Sometimes only one taster was available and never did more than three take part. The history of some lots tasted and taster's preferences are shown in Table 2. The writer often took part in these tests but his reactions are not recorded because he knew the history of the coded groups and felt he could not do the tasting objectively. In almost all cases his reactions were in accord with those reported. The tasters' reactions were scored according to the rating scale used in the first experiment.

Remarks made by tasters are especially significant where such small numbers of observations are concerned and these are tabulated along with preference votes.

SHELL EROSION DURING STORAGE

Dugal (1939) reported that inner faces of shell of quahaugs are eroded (etched) during air storage by acid products of anaerobic respiration. The amount of etching increased with storage time and was more rapid at higher temperatures. Kuhnelt (1938) showed that valves of European oysters, O. edulis, may suffer similar erosion but to a lesser extent. Their glossy inner shell faces acquire a dull chalky appearance. It is believed that storage taste of oysters also results from accumulated products of anaerobic respiration or compounds of these with the lime of the shell. It seemed possible,

Table 2. Development and clearance of storage flavour, as detected by varying numbers of tasters, in paired groups of oysters after various treatments during the winter of 1942-1943. The bracketed numbers in the right-hand column are taste scores such as were used in Table 1.

Test No.	Paired groups tasted	History of groups	No. of tasters showing preference	Tasters' remarks and taste scores
1	(a)	Directly from cold tank	2	All good (100)
	(b)	Cold tank to air 4 days at 60°F.	0	Pleasant but inferior (90)
2	(a)	Directly from cold tank	3	All good and sweet (100)
	(b)	Cold tank to air 5 days at 60°F.	0	Lost sweetness; storage taste detectable (90)
3	(a)	Directly from cold tank	3	All good (100)
	(b)	Cold tank to air 10 days at 60°F.	0	Distinct storage taste (75)
4	(a)	Directly from cold tank	3	All good (100)
	(b)	Cold tank to air 2 months at 60°F.	0	Unpalatable (25 to 50)
5	(a)	Cold tank to air 5 days at 60°F. then to water 2 days at 60°F.	1	Good (100) Little difference; no obvious storage taste
	(b)	Cold tank to water 2 days at 60°F.	1	Good (100)
6	(a)	Directly from cold tank	1	Preference slight (100)
	(b)	Cold tank to air 5 days at 60°F. then to water 2 days at 60°F. then to cold tank 5 days	0	No storage taste (90 to 100)
7	(a)	Sampling of stock in cold tank after 4 months		No storage taste (100)

therefore, that our oysters might show shell etching and that its extent might be useful, together with flavour, as indices for judging storage age of oysters and hence their eating qualities and potentialities for further storage without mortalities or undue deterioration in flavour.

To examine this possibility observations were made on shells of several samples of Cooper Bed oysters gathered on various dates and stored for various periods before condition tests. Besides this, observations were made on shells of those oysters brought to St. Andrews in November 1942, and air-stored at 45°F. in the basement of the Biological Station.

Records were made of the frequency of etched oysters in the samples studied and of distribution of etching in affected animals. Observations were recorded by fractional formulae. If 8 oysters in a sample of 20 showed etching of one or both shell valves, the etching frequency for the sample was recorded as 8/20. The frequency and distribution of etching of valves in such a sample were variously recorded. If both valves of all 8 showed etching, the distribution was recorded as 16/40. If the etching of some of the 8 affected oysters involved only one valve, then a formula such as 12/40 might have been used indicating that in a total of 40 valves, 12 were etched. Sometimes the distribution in upper and lower valves was recorded. In such cases the formulae used were more complex. For example, 12(4U8L)/40 would indicate that, of the 12 etched valves, 4 were uppers and 8 were lowers. Table 3 describes some of the samples studied.

DISCUSSION OF RESULTS

Survival and Drying

Figure 1 indicates that survival was best when oysters were left in water on their native beds. There were no obvious losses. In air storage mortalities were considerable and came earlier and were greater at higher storage temperatures. Reading from the graph (Fig. 1) for the highest temperature tested 48 to 53°F., simulating conditions in a warm cellar of a centrally heated house, the loss approximated 50% after 2 months and 100% after 4 months. At temperatures just above freezing, such as obtain in commercial cold storage houses, losses were apparently not serious until after 3 months. They approached 50% at 4 months.

It is impossible to say what killed the oysters. Perhaps they were poisoned by the accumulation of their excretions but they could have died simply from drying. Figure 2 shows that drying does go on at a rapid and steady rate during air-exposure. Furthermore, stored oysters used in mid-winter and late-winter condition tests had lost much of their shell liquor. The shells themselves are almost

Table 3. Etching of inner faces of Cooper Bed oysters variously treated.

Date fished	Period of air storage before examination (days)	Frequency of etched oysters in sample examined	Distribution of etching in valves	Remarks
26 July 1940	2	0/10	0/20	Shell faces glossy with exposed chalk
10 Sept. 1940	2	0/10	0/20	"
10 Nov. 1940	3	4/8	5/16	Shell faces in "hard-flat" condition
1 Dec. 1942	44	5/9	$5(5L0U)/_{18}$	"
"	60	5/7	$8(5L3U)/_{14}$	"
"	98	10/10	$16(9L7U)/_{20}$	Etching conspicuous
26 Jan. 1943	4 (hours)	13/23	16/46	Fished through ice; etching slight

impervious (Medcof 1944) and I agree with Fingerman et al (1957) that this loss results from evaporation of shell liquor slowly escaping from the shell margin.

Needler (1934) suggested that air-stored oysters occasionally open their shells and advised that for extended storage they should be packed with the cupped shell down to prevent shell liquor from draining away. Mr. R. Drinnan (personal communication) states that he has kymograph records of ever-so-slight exploratory opening of shells of air-stored European oysters and that this opening was so slight that it would probably escape visual observation. If air-stored oysters do indeed open their shells it is understandable that they might dry quickly. Regardless of whether they open their shells, all agree that they do dry out. Fingerman et al (1957) showed that drying has remarkable effects on meat yield and our cold storage operators believe it kills oysters. It will kill most animals.

The presence of gas bubbles in the hearts of air-stored oysters could mean that drying kills them by impeding or preventing blood circulation. However, Lund's ingenious experiments (1957) show that oysters that are artificially kept closed in sea water, where no drying is possible, also die in a short time. He further showed that the damaging effects of enforced closure become irreversible quite some time before actual death. As a result his oysters died soon after they were allowed to open. My own experience and that of two of my colleagues, Drs. Logie and Leim (personal communications) show that similar irreversible changes must also occur in oysters that are air-stored for lengthy periods. Although still alive, they soon die when placed in normal sea water.

It must be admitted that evidence is not clear but I am inclined to the belief of cold storage operators that mortalities during air storage are largely attributable to drying.

Index of Condition

Figure 1 shows that index of condition of oysters left on their beds does not change remarkably during cold months. This accords with observations reported earlier (Medcof and Needler 1941). Apparently autumn-stored glycogen which is the major organic constituent (Baker et al 1947) is normally reserved for gonad development the following spring and not used in substantial amounts to sustain the animal during its winter sleep as a bear uses its fat.

In air storage, however, the index did fall at all storage temperatures. High consumption of carbohydrates in anaerobic respiration and loss of water and dissolved salts could produce the observed thin flaccid condition of the meats of these oysters.

Possibly both processes took place during air storage in the basement and sub-basement and either would reduce the quality of oysters for half-shell trade.

Flavour

Flavour (smell and taste) of oysters hibernating on their beds varied (Fig.1). In mid-winter they had a faint storage taste. This taste is variously described as "metallic" or "musty" depending on the length of the storage period. Its appearance suggests that anaerobic respiration goes on in mid-winter at temperatures about 30°F. Loosanoff (1958) reports that most oysters do not pump at temperatures about 32°F. The appearance of the storage taste at 30°F. indicates that the oysters accumulate the products of respiration and may even be respiring anaerobically. It is hard to believe that flavour would be affected if the shell cavities were well flushed out. However, the fact that Buctouche winter-fished oysters are held in such high regard and the fact that the mid-winter sample of Cooper Bed oysters had only a faint storage taste (Table 1) suggests that this accumulation of waste products is much less in cold water than in air storage. By early May, when the water temperature had risen slightly but was still in the 30's and low 40's, the storage taste had disappeared and this suggests that occasional opening of shells and pumping in cold water (Loosanoff 1958) permitted the escape of metabolites of anaerobic respiration.

Artificial storage in tanks of cold circulating sea water (Table 2, test 7) seems to provide much the same conditions as are found in nature.

Figure 1 also shows that flavour deteriorated seriously (score dropped to 50) but not to the point of unpalatability after three months' air storage when temperatures were favourably close to 32°F. At temperatures corresponding to those of a cool or a warm house cellar (cf. basement and sub-basement of the Biological Sub-Station) flavour degenerated to levels of unpalatability in less than half this time. These off-flavours probably resulted from accumulation of metabolites of anaerobic respiration except near the end when the animals were dead, or nearly so, and putrifaction may have been responsible.

The design of the experiments reported in Figure 1 permitted detection of only gross changes in flavour but the experiments on development and clearance of storage flavour were more sensitive and showed that flavour is affected by as little as 4 or 5 days' air storage at 60°F. This accords with Dugal's observation (1939) that the carbon-dioxide content of the shell liquor of quahaugs rises within 4 days to a high level approximating plateau levels that are maintained during long periods of storage. After 10 days the taste score for our oysters air-stored at 60°F. had dropped to 75 and

after 2 months they were barely edible. However, this storage taste disappeared completely from those animals which were still sound when they were placed in water at temperatures of 60°F. which encourage pumping and even at temperatures below but close to 40°F.

Apparently the animals can be switched back and forth from air to water (Table 2, tests 5 and 6) without impairing their ability to free themselves of storage taste when returned to sea water except that after very long air storage some sort of irreversible change occurs and they die as already described. This accords with Dugal's observations on quahaugs. When his air-stored animals were returned to warm salt water (temperature not stated) the high carbon-dioxide content of their shell liquor fell to normal levels within a few hours.

Shell Erosion

The results in Table 3 are interesting from several points of view. Kuhnelt (1938) observed that shell erosion appears in Mytilus after one day's air exposure but that it was not apparent on glossy parts of the shell of Ostrea edulis even after long exposure when they carried exposed layers of chalky deposits. I have described similar chalky deposits in our own species during the summer season (Medcof 1944) and it is not surprising that there was no apparent erosion in the July 26 and September 10 samples (Table 3). It seems that when chalky areas are present they alone are eroded and that they are so lacking in character that it is impossible to tell whether they have been attacked.

Almost all the other samples studied were fished in autumn or winter and had developed the hard, flat-white surface characteristic of oysters fished in those seasons (Medcof 1944). Etching of the sort Kuhnelt has described (1938) of such surfaces would be practically undetectable. However, etching of a kind did appear in some shells. These showed irregularly-shaped areas usually less than 1 cm.^{2} outlined by faint, crinkly lines. I have interpreted these as an extreme development of the erosion described by Kuhnelt. They were seldom found anywhere except anterior to the muscle scars in central parts of lower valves. It was obvious that a surface layer of lime had been removed. A sample of these was sent to Dr. Dugal in January 1943 who reported (personal communication) that this erosion was of the sort he had observed in quahaugs but much less pronounced.

The November 10, 1940, sample (Table 3) showed some etching although the oysters had been out of water only 3 days. The water temperature at that time was ranging about 41°F. when one would expect some pumping (Loosanoff 1938) and no anaerobic respiration. It seems safe to assume then that all this erosion (only the oysters were affected in only one valve) took place during their air exposure.

Other samples fished in November and opened within 3 or 4 hours showed no erosion. Frequency of erosion in the 3-day-old sample was only slightly less than that in oysters that were air-stored 44 and 60 days (Table 3). However, almost all oysters stored 98 days showed conspicuous erosion and both valves were usually affected.

About half the oysters fished through the ice on January 26, 1943, and opened within 4 hours (Table 3) showed erosion. The water temperature then varied about 29°F. Remembering the erosion-free condition of freshly-fished November samples it seems safe to assume that the January oysters had been respiring anaerobically to some extent at least. This observation accords with that of February 23, 1940, reported in the first experiment (Table 1). Oysters fished through the ice at that time had a faint storage taste. If their shells had been examined it seems likely that they too would have shown erosion.

The general conclusion is that in our oysters, shell erosion produced by anaerobic respiration during air storage is too vague to be useful as an index to storage age, eating quality and potentialities for further storage. It is conspicuous only in animals that are approaching the limits of potential storage periods.

Mr. R. Drinnan has suggested that the calcium content of the shell liquor might be useful as an index. Determining calcium content would be considerably more involved than simple shell examination but it might have merits. It should be explored.

Practical Implications

Whether we like it or not our climate obliges us to store oysters for the half-shell trade and these experiments substantiate claims of producers, dealers, and cold-storage operators that present methods of storage are unsatisfactory. In the first place they are expensive -- rates are necessarily high and often storage periods are long. Besides this they often involve costly mortalities, especially in the upper parts of storage containers (barrels and boxes) where drying is worst. Then too, the frequent long delays between shipment to cold storage and eventual sales force many producers to dispose of their oysters through brokers on consignment which is not always to the producers' financial advantage. Recently, some cold-storage operators have refused to handle oysters in the shell. They prefer to deal in products that have a quicker turn-over. This poses a critical problem for producers. New and better storage methods must be sought at once.

The experiments also confirm the claims of many consumers who say that winter oysters are not good oysters. Some consumers who are accustomed to eating stored oysters, have no objection to storage flavour but most consumers dislike it. If producers are to expand

markets when our oyster stocks recover from the current epidemic disease, they must take fuller account of consumer preferences. People don't "have" to buy oysters. Oysters are a luxury food and consumers need not tolerate poor quality in a luxury food as they are sometimes obliged to tolerate it in staple foods. Obviously then, industry should search for new storage methods that will circumvent not only their own immediate economic problems but also provide better eating qualities of their product which in the long run should indirectly afford them the advantages of increased markets. Judging from our experiments, water storage during winter may be what is needed. We have shown that oysters held for 4 months in circulated cold sea water developed no storage taste and suffered negligible mortalities.

Europeans who handle the flat oyster (Ostrea edulis) which cannot withstand lengthy air storage, have developed satisfactory methods of water storage. Mortalities are low for there is no drying. The producer holds his oysters at the coast until they are ordered by dealers at arranged prices. They need not ship on consignment and be victimized by the vagaries of markets. The consumer, on the other hand, buys oysters in mid-winter (or even in late winter and early spring) that are as free of storage taste as those he buys in autumn. For him the oyster season is long. Indeed, as the old saying goes, it includes every month with an "r".

We may not be able to copy European methods but we certainly should explore possibilities of imitating them.

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PREDICTION OF OYSTER SETTING IN BRITISH COLUMBIA (CRASSOSTREA GIGAS)

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Abstract

Erratic reproduction of the introduced Japanese oyster (Crassostrea gigas) has created a need for spatfall predictions in British Columbia. An empirical method of determining time and approximate intensity of setting is described.

The Pacific oyster (Crassostrea gigas) is not indigenous to the Pacific Northwest where summer water temperatures are considerably lower than in its native habitat in Japan. Breeding is therefore uncertain except in a relatively few areas, where unique hydrographic conditions permit temperatures to attain required levels. Even here, due to variable summer weather in the Pacific Northwest, in some years breeding temperatures are not reached or maintained for long enough periods. Consequently there may be neither spawning nor setting. When spawning does occur, larval broods may not survive. In contrast to the long breeding period of Crassostrea virginica in Virginia and adjacent areas, larval broods on the west coast are generally discrete and setting is often restricted to a few days.

Since spatting is erratic and timing important to avoid fouling, blind cultching is both impractical and uneconomical. Therefore spatfall prediction is desirable and collection of oyster seed in Washington and British Columbia is based largely on spatfall forecasts.

In British Columbia, only in Ladysmith Harbour and Pendrell Sound, both relatively small areas, does setting occur with relative frequency and it is here that efforts to predict have been concentrated (Quayle 1955, 1949-57). The technique of prediction is essentially the same in both areas but hydrographic differences cause variation in both breeding and forecasting success.

Prediction Technique

Oyster spawning in British Columbia is usually sudden and complete, and is often observed visually. Therefore individual larval broods may be followed closely with respect to number and distribution of larvae, by means of both spot and running plankton samples.

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In Pendrell Sound, for example, in an area of five square miles, 14 spot samples of one to three cubic feet taken three feet below the surface at intervals of not more than three days, have been found to describe, adequately for prediction, trends in numbers of larvae. Although the number of advanced-stage larvae per unit volume of water is the ultimate basis for prediction, rate of reduction in number is also of importance. Trends in water temperature and weather conditions and their effect on final abundance of larvae must also be considered. Low water temperatures caused by rain or cloud cover frequently coincide with diminution of larval broods or even their decimation.

Spawnings are reported to the industry as soon as straight-hinged larvae are observed in the plankton. The number of larvae in the immediate vicinity of the spawning is used to make a preliminary estimate of the possibility of a commercial set. Reports are provided at about weekly intervals and information is made available through a regular Fisherman's Broadcast as well as by printed bulletins.

The larval period of the Pacific oyster may vary between 18 and 30 days depending on temperature and possibly other factors (Quayle 1954). By following size distribution of larvae and water temperature it is possible to modify predictions of date of beginning of setting. Prediction becomes more definite as the brood progresses and the final decision is given about a week before the expected date of the set.

It is apparent that the method of prediction is purely empirical. Each breeding area has its own characteristics and reliability of prediction is increased by experience.

Insufficient data have been accumulated to indicate accurately numbers of straight-hinged larvae required for a minimum commercial set under optimum conditions, but for Pendrell Sound it appears to be about 10 to 25 per gallon. In both areas, about one advanced-stage larva per gallon is required to produce a set of at least ten spat per shell.

Ladysmith Harbour

Ladysmith Harbour is a narrow, fairly-shallow bay, somewhat different from the usual fjord form of British Columbia inlets.

Summer salinities lie within the range 23 to 26 ppt and surface temperatures within the limits 60° to 68°F. Heating and cooling of tidal flats in the harbour contributes materially to rapid and considerable temperature changes. Temperature structure may also be quickly changed by wind mixing. A two-layered current system has been demonstrated, and a significant advection of phytoplankton into the inner harbour occurs (McAllister 1956). This type of circulation

no doubt aids in retention of oyster larval populations, all of which originate normally within the harbour.

Formerly, spawning in Ladysmith Harbour was quite irregular, and between 1930 and 1940 stimulation on a large scale was practiced (Elsey 1936), but more recently natural spawning has occurred quite regularly. There have been occasions, however, when extensive larval mortality occurred, in spite of what appeared to be satisfactory temperature conditions.

In Ladysmith Harbour, commercial spatfalls (a mean of 10 spat per shell) have occurred on floating cultch in only eight of the last 24 years. Spatfall predictions based on quantitative larval sampling have been made since 1949. The record of forecasting success has not been particularly good in Ladysmith Harbour for only six of ten forecasts may be considered satisfactory in respect to both time and extent of setting. Because of hydrographic conditions, Ladysmith Harbour will no doubt continue to be a difficult area in which to predict. More intensive sampling would probably increase efficiency.

In spite of the rather poor record from the biological point of view, forecasts in this area have been relatively satisfactory for growers who collect seed there, for timing has been right. On only one occasion has the prediction caused the industry to miss part of a set.

Pendrell Sound

Pendrell Sound is a typical fjord-type inlet with steep rocky shores and depths up to 1,000 feet. Natural oyster populations occur on the steep rocky walls of the sound. During early summer a layer of relatively fresh water (20 ppt) about 15 feet deep is formed over the main water mass of salinity about 30 ppt. It is in this thin surface layer, in which temperature may reach 75°F., that larval broods occur (Quayle 1957).

Surface currents are extremely weak and seldom exceed 1/4 knot. There is no sill at the entrance to this inlet to create turbulence, and main current action is along the bottom. Prevailing summer winds do not destroy stratification which disappears only with the advent of autumn storms.

In only one of nine years in which the area has been studied has the average spatfall on floating cultch been less than ten per shell and in four it has been well over 100 per shell. Of ten predictions made for this area up to 1956, one may be considered unsatisfactory.

Discussion

In Ladysmith Harbour where only local growers collect seed, ten spat per shell is considered the "commercial" level, although growers will cultch for less. In Pendrell Sound where operations are carried on by seed producers, the "commercial" level is about 25 spat per shell. Prediction is required to indicate whether these levels will be reached.

When weather is good, water temperatures average or above, and larval abundance high, there is little difficulty in predicting a commercial set. The difficulty is increased if larval numbers are low. When weather conditions are unfavorable (rain and cloud cover), prediction is difficult regardless of larval numbers.

The possibility of reducing prediction of Pacific oyster spatfalls in British Columbia to a mathematical basis is unlikely, for both initiation of spawning and larval survival appear to depend on water temperature which is largely a function of weather conditions in the breeding areas. Each larval brood must be considered as an individual case.

The plankton sampling and counting required in prediction studies involve considerable effort, but the return in efficiency of seed collection makes it well worth while in British Columbia.

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PACIFIC OYSTER SEED PRODUCTION (CRASSOSTREA GIGAS)

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Abstract

Procurement of seed oysters (Crassostrea gigas) needed to sustain the Pacific oyster industry in the Pacific northwest is described. The two main sources together with the type of seed and cultch from each are compared.

Utilization of Oyster Seed

Supply of seed for the Pacific oyster industry has not been a very serious problem in the past. The increasing cost of seed, materials and labour relative to the unchanging price of market oysters requires a serious review of supply and utilization of seed oysters.

In discussions of seed oysters one significant point is often overlocked, and this is compatibility of the seed and the culture for which it is intended.

There is an almost infinite number of types of oyster culture although it is an exaggeration to apply the term "culture" to many current methods of growing oysters. In some cases the culture is adapted to the type of seed; in others, the seed is adapted to the type of culture. Contrasted to variations possible in seed, ecological factors that characterize a given oyster bottom are relatively fixed. Seed is more easily adapted to the method of culture than the reverse. The type of cultch, and therefore seed, may be varied to fit the culture.

Pacific-Oyster Seed

Japanese Seed. Pacific-oyster breeding in the Pacific Northwest was originally quite erratic. Consequently the industry came to rely on annual imports of Japanese seed that was collected especially for this export trade. The spat were of quite uniform size and age. This permitted individual year classes to be grown as a unit, so at harvest the oysters were relatively uniform in size and quality. Separate growing and breeding areas on the West Coast assist in developing this type of uniformity.

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Except for the war years, the relatively stable supply of Japanese seed and continued low cost created little incentive to develop alternatives, although dependence of the industry on a single source is hazardous. In addition, Japanese producers have been willing to change the type of seed according to the wishes of U. S. growers.

Scallop shells were used initially but found to be unsatisfactory. Subsequently whole oyster shell ("unbroken") became the standard cultch, then broken oyster shell, which gave both a greater number of spat and of cultch pieces per case. Increased spat count, in addition to the concept that smaller clusters would not require separation, has made broken oyster shell a very popular form of seed. The industry failed to appreciate that smaller pieces of cultch led to increased mortality from silting and that there were just as many clusters requiring separation. On the average, the industry received little advantage from the more costly broken seed, with standard methods of culture. Broken seed would have had definite advantages and benefits if the culture had been adapted to the new form of seed by placing it on ground to which it was suited. Instead, a very considerable part of each year's seed was literally buried in the bottom.

The most recent change has been the development of high-count seed, where spat count per case was tripled over unbroken seed and nearly doubled over broken. High-count seed may be obtained only by making the pieces of mother shell smaller or by increasing the spat count, the former being the most important method. The result of this move was a further increase in silting mortality, again because cultural techniques were not adapted to the new type of seed. On the basis of experiments on seed with quite high counts (50-100 thousand per case), mortality was about 90% between planting and marketing, although absolute survival was greater than with standard broken seed under slightly better than average bottom conditions. Only because of the unique price structure of Japanese seed has it been profitable to use high-count seed.

Local Seed. The alternative to the Japanese supply is locally-produced seed. Several factors have militated against its greater use in the past. These are the relatively low cost (up to now) of Japanese seed, inconsistency of local supply, and type of shell cultch. The cost of Japanese seed is rising rapidly and is almost certain to increase much faster than the cost of local seed. Consistency of supply by local breeding has improved in recent years. The probability of receiving commercial sets in Dabob Bay (Washington) on floating cultch is at least 75% certain (Quilcene Lab. Report, 1954). In Willapa Harbour between 1936 and 1958 there have been only 7 years when setting has been non-commercial (less than 3 per shell) (Sayce 1957). In Pendrell Sound, B. C., spatfall has fallen below an average of 10 per shell only in one year during the period 1949-1958 (Quayle 1955, 1957). It is unlikely that all areas will fail in any given year, so the possibility of a reasonably consistent supply of local

seed exists. On the other hand, shell is still the basic cultch for local seed and its deficiencies are many and obvious. Heavy shell is considered by industry to be a very serious drawback.

For collecting local seed there are three basic plans, all of them based on spatfall prediction. The first is bottom-spread shell cultch. This is an inefficient method and its deficiencies stem from two main factors:

1. If the shelling operation is to be on a large scale, spreading must begin the moment occurrence of a larval brood is reported. If this fails to survive, and frequently there may be only one significant brood per season, the shell is lost.

2. Even with excellent spatfalls, low efficiency of bottom-spread cultch renders its use doubtful, both practically and economically, in comparison to results obtained from prepared collectors such as shell strings or shell bags. The low efficiency is mainly due to fouling. In Table I is given spatfall-per-shell frequency for the 1957 spatfall in Willapa Harbour, Washington, when strings hung from intertidal racks caught well over an average of 100 spat per shell. This table shows that 40% of the loose shell held no spat, and another 13% held between one and three spat per shell. These counts were made in September on approximately 11,000 shells.

The second basic plan is to use intertidal racks from which shell strings or shell bags are hung. This is much more efficient than bottom-spread cultch. Preparations may be made well in advance and exposure may be delayed until the probability of a set has become high. In event of a set failure the cultch may be recovered. Growth rate of spat is high and mortality is relatively low. With low larval production rack cultch will collect a significantly greater spatfall than bottom cultch.

In the third plan, rafts are used to suspend either shell strings or bags. While generally more costly than the other two methods, efficiency is greater and commercial sets may be obtained when little or no spatfall is obtained by the other methods. Growth rate is also higher and some labor problems might be solved for little attention need be given to tidal conditions. Oil drums or log rafts may be used. Creosoted plywood pontoons, while initially expensive, would be highly efficient for they could be used for other purposes or stored ashore, and would last indefinitely.

Cultch

No discussion of seed oysters would be complete without mention of cultch problems. The most serious drawback in oyster seed production today is the cultching medium. As a specific cultch, shell has many deficiencies which include great weight and strength, which with heavy spatfall create large clusters whose separation is difficult, although not impossible. The chief attributes of shell are

availability and low cost. Many alternatives have been proposed, but all have failed to approach even closely the requisites of an ideal cultch. One of the most significant forward steps possible in oyster culture today would be development of a satisfactory cultch.

Summary

The future trend of oyster seed production on the Pacific Coast will lie in greater use of local seed. This is inevitable because of increasing cost of Japanese seed and realization that dependence on a single seed source over which industry has little or no control is not good business. The trend would be hastened considerably by development of a suitable cultch.

Table I. Spatfall of Crassostrea gigas on loose shell, Willapa Harbour, Washington, September, 1957.

Spatfall per shell	Percentage occurrence			
	Coon Rock	South Dock	Naselle Bay	Average
0	43	41	37	40
1-3	10	16	13	13
4-6	7	9	10	9
7-9	5	7	8	7
10-12	5	5	6	6
13-15	3	5	4	4
16-18	3	3	3	3
19-21	2	2	3	3
22-24	1	1	2	2
25-27	4	4	2	3
28-30	2	1	2	1
31-33	2	1	1	1
34-36	1	1	1	1
37-39	1	1	1	1
40-42	1	1	1	1
43-45	1	1	1	1
46-48	1	0	0	1
49-51	5	1	2	2

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INTERPRETATION OF OCEANOGRAPHIC MEASUREMENTS - THERMODYNAMICS

by

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INTERPRETATION OF OCEANOGRAPHIC* MEASUREMENTS - THERMODYNAMICS

*N. P. Fofonoff***

HISTORICAL DEVELOPMENT

Much of the early interest in the physical properties of sea water was developed through efforts to determine accurate subsurface ocean temperatures. Thus the history of the development of thermometers for use at sea is closely linked with the study of thermodynamics of sea water.

The earliest attempts to measure deep ocean temperatures were carried out in the 18th century. Two methods were used. One consisted of raising a sample of water in an insulated container and measuring temperature on deck. No corrections were applied to compensate for temperature changes due to adiabatic decompression of the water sample. Indeed, the correction for this effect, had it been known, would have been trivial compared with errors introduced by raising the sample through the warmer surface layers where heat conduction and leakage could alter the temperature considerably. This method continued to be used, with improvements, even in the early part of the twentieth century and is still used to obtain surface temperatures.

The second method was to lower a thermometer encased in insulating material (wax) to the desired depth, allow it to come to equilibrium and haul it in quickly to read the temperature. Helland-Hansen (1908) credits De Saussure with the first series of deep ocean temperatures taken in 1780 in the Mediterranean Sea using this method.

Both methods were unreliable, as were the early thermometers, and errors were of the order of degrees. Nevertheless, these early measurements served to determine the general features of temperature distribution in lakes and the oceans.

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Advances in thermometer design came and early in the nineteenth century thermometers with minimum and maximum indices were put into use. The earlier versions were not protected and corrections to eliminate the effects of pressure had to be applied. The pressure calibration was far from uniform even for identical makes of thermometers. Each thermometer had to be calibrated individually in a pressure tank. Thermometers were often lost or broken in use before being calibrated. The result was that a good deal of conflicting deep-water temperature data was gathered.

In the absence of reliable deep-water temperature measurements many scientists were convinced that the deep ocean maintained a uniform temperature of 39°F (the density maximum of fresh water). This belief persisted even though Despretz in 1837 showed that sea water did not exhibit a density maximum with temperature above its freezing point.

Unprotected thermometers were used as late as 1868 although by this time abundant evidence as to their unreliability was available. Protected thermometers came into use in 1858, although they were apparently used in 1818 on the Arctic expedition headed by Sir John Ross. They were a great improvement over the earlier thermometers. The inner bulb of the thermometer was enclosed by an outer bulb to take up the pressure. The space between was partially filled with alcohol or mercury to provide more rapid heat conduction. The stem was not protected and usually no correction was applied for stem compression.

Protected thermometers were used on the Challenger expedition (1872-1876) for almost all temperature determinations, although towards the end of the cruise reversing thermometers were tried out with limited success. These early reversing thermometers, built by Negretti and Zambra, had to be rotated through a complete turn to obtain a reading. Most of the shipment to the Challenger was broken in transit, but several thermometers arrived intact and were used. However, they did not compare in accuracy with the min-max thermometers.

The major failing of the min-max thermometers was that they were incapable of giving the temperature distribution under an inversion. Prior to the Challenger expedition, the optimistic belief was that such inversions did not occur in the ocean. The min-max thermometers were eventually superseded by fully-protected reversing thermometers of improved design. Meanwhile, unprotected reversing thermometers had been developed into quite sensitive pressure gauges.

Electrical resistance thermometers have been developed and tested from time to time, but they have never exhibited sufficient stability and reliability to come into wide usage. Resistance thermometers were in use prior to the Challenger expedition.

Early interest in compressibility and adiabatic temperature changes of sea water was stimulated largely by efforts to calibrate thermometers. Pressure tanks had to be built for the calibrations and these were used to determine properties of sea water under pressure. Curiously enough, the first measurements of compressibility of fresh and salt water were reported by Canton (1762) in a paper entitled "Experiments to prove that water is not compressible." His experiments indicated that compressibility decreased with temperature and in another paper he calculated that sea level is lower by 69 feet in two miles because of compressibility of sea water. Perkins (1826) showed that compressibility decreased with pressure.

The first extensive series of compressibility measurements of sea water were performed by Prof. P. G. Tait (1889) using the pressure tank constructed to check the Challenger thermometers. He found that compressibility decreased with temperature and pressure. Both these facts, although first demonstrated much earlier, were still controversial in his day.

Although the correct general features of the equation of state of sea water were established in the nineteenth century, the measurements on which present knowledge is based were carried out by Knudsen (1901) and Eckman (1908). No recent verification of these determinations have been made, with the exception of density at 0°C (Thompson and Wirth, 1931).

Other properties of sea water have received much less attention. Freezing point and vapor pressure have been measured, but specific heat, heat of dilution, latent heats of fusion and evaporation and properties such as viscosity, thermal conductivity and diffusion have not been adequately investigated. An excellent source of reference and discussion of the earlier work on sea-water properties is given by Dorsey (1940).

A summary of the sources of present knowledge of the physical properties of sea water is given in Table 1. The table also includes some earlier works of historical interest.

TABLE 1

A SUMMARY OF SOURCES OF PRESENT KNOWLEDGE OF THE PHYSICAL PROPERTIES OF SEA WATER

Density of sea water at atmospheric pressure	C. Despretz	1837	Showed that water does not have density maximum above freezing point.
	M. Knudsen	1901	Prepared tables of sea-water density at atmospheric pressure based on his measurements.
	T. G. Thompson H. E. Wirth	1931	Checked Knudsen's determination of density at 0°C and found it satisfactory.
Compressibility	Canton	1762	First to show water is compressible.
	P. G. Tait	1889	First extensive series of compressibility measurements
	V. W. Ekman	1908	Measurements used in present tables.
Specific Heat	J. Thoulet A. Chevallier	1889	C_p measured at 17.5°C.
	R. A. Cox	1958	Complete determination of C_p at atmospheric pressure.
Freezing Point	M. Knudsen	1903	Measured temperature of equilibrium of pure ice with sea water.
	Y. Miyake	1939	
Vapor Pressure	R. Witting	1908	Calculated from freezing-point depression.
	K. Higashi K. Nakamura R. Hara	1931	Vapor pressure of sea water at high temperatures 25°C to 175°C.
	A. B. Arons C. F. Kientzler	1954	Vapor pressure in range -10°C to 35°C.
Viscosity	O. Krummel	1907	Decreases rapidly with temperature and increases slightly with salinity.
Thermal Conductivity	O. Krummel	1907	Computed from assumption $(k/\rho c_p)_{\text{sea water}} = (k/\rho c_p)_{\text{pure water}}$.

TABLE 1

A SUMMARY OF SOURCES OF PRESENT KNOWLEDGE OF THE PHYSICAL PROPERTIES OF SEA WATER—Continued

Electrical Conductivity	E. Ruppin	1906	Specific conductance increases with temperature and salinity.
	B. D. Thomas T. G. Thompson C. L. Utterback	1934	
	W. Bein H. G. Hirsekorn L. Moller	1935	
Surface Tension	O. Krummel	1907	Higher than pure water at same temperature.
Refractive Index	O. Krummel	1907	Increases with temperature and salinity.
	C. L. Utterback T. G. Thompson B. D. Thomas	1934	
	W. Bein H. G. Hirsekorn L. Moller	1935	
	Y. Miyake	1939	

THERMODYNAMICS OF A SEA-WATER SYSTEM

The ocean forms a complex thermodynamic system in a gravitational field. Because of the increase of pressure with depth, it must be considered as consisting of infinitely many phases in its vertical extent. These phases are not, in general, in equilibrium with each other so that there is a continuous transfer of heat and mass between adjacent phases.

The thermodynamic state of a particular phase is described by measuring the pressure, temperature, and salinity. These three independent variables are then used to compute the depth, i.e., the location of the phase relative to the gravitational potential field, and other physical and thermodynamical quantities.

The changes of other thermodynamical quantities such as internal energy can be described starting with a general statement of the first Law (cf. Guggenheim "Thermodynamics," p. 356).

$$dU = Td\eta' - pdV + \Sigma(\mu_i + \Phi)dm_i \qquad (1)$$

with

U = internal energy

T = absolute temperature

η' = entropy

p = pressure

V = volume

μ_i = partial chemical potential of the i-th constituent

Φ = gravitational potential (geopotential)

m_i = mass of the i-th constituent.

Equation (1) describes the changes of internal energy of a general system. It can be converted to apply to a unit mass of sea water by substituting into (1) the following intensive quantities:

$u = U/M$ specific internal energy,

$\eta = \eta'/M$ specific internal entropy

$\alpha = V/M$ specific volume,

$s = S/10^3 = \dfrac{\Sigma m_i - m_\omega}{M}$, salt content per unit mass (salinity), where m_ω is the mass of water in the total mass M at salinity S,

$\mu_s = \Sigma \mu_i \lambda_i$, effective partial chemical potential of the dissolved salts, assuming that the i-th solute constituent bears a fixed ratio λ_i to the salinity so that $m_i = M\lambda_i S$,

μ_ω = partial chemical potential of water, and

$$\mu = \mu_s - \mu_\omega .$$

The substitution yields, assuming M and dM to be independent,

$$du = Td\eta - pd\alpha + \mu ds \tag{2}$$

$$u = T\eta - p\alpha + \mu s + \mu_\omega + \Phi \tag{3}$$

and the Gibbs-Duhem relation

$$d\mu_\omega + d\Phi = -\eta dT + \alpha dp - Sd\mu \tag{4}$$

These equations can be applied only to those processes which do not change the ratio of the dissolved salts.

Other thermodynamic quantities such as specific enthalpy h, specific free energy f, and the thermodynamic potential g are defined in the usual way, i.e.,

$$h = u + p\alpha$$

$$f = u - T\eta$$

$$g = u - T\eta + p\alpha$$

and are considered as functions of temperature, salinity and pressure.

A long list of relationships between the thermodynamic variables can be derived by means of simple differential transformations. Among the more interesting of these are:

$$\left(\frac{\partial \eta}{\partial p}\right)_{T,s} = -\left(\frac{\partial \alpha}{\partial T}\right)_{p,s} \tag{5}$$

$$\left(\frac{\partial \eta}{\partial s}\right)_{T,p} = -\left(\frac{\partial \mu}{\partial T}\right)_{p,s} \tag{6}$$

$$\left(\frac{\partial \mu}{\partial p}\right)_{T,s} = \left(\frac{\partial \alpha}{\partial s}\right)_{T,p} \tag{7}$$

These relations can be differentiated again to yield

$$\left(\frac{\partial C_p}{\partial p}\right)_{T,s} = -T\left(\frac{\partial^2 \alpha}{\partial T^2}\right)_{p,s} \tag{8}$$

$$\left(\frac{\partial C_p}{\partial s}\right)_{T,p} = -T\left(\frac{\partial^2 \mu}{\partial T^2}\right)_{p,s} \tag{9}$$

where $C_p = T(\partial\eta/\partial T)_{p,s}$ is the specific heat at constant pressure.

The thermodynamic properties of sea water are completely described if the internal energy is known (Equation 2) as a function of entropy, specific volume, and salinity, or alternatively if the thermodynamic potential g is known as a function of temperature, pressure and salinity.

If g is known, then

$$\left(\frac{\partial g}{\partial T}\right)_{p,s} = -\eta$$

$$\left(\frac{\partial g}{\partial p}\right)_{T,s} = \alpha$$

$$\left(\frac{\partial g}{\partial s}\right)_{T,p} = \mu$$

Thus, a complete thermodynamic description requires knowledge of the entropy, specific volume (equation of state) and the partial chemical potential as a function of temperature, pressure and salinity.

ENTROPY

The entropy of sea water at atmospheric pressure is determined from the equation

$$d\eta = \frac{c_p}{T}\,dT + \left(\frac{\partial \eta}{\partial s}\right)_{T,p} ds \tag{10}$$

Neither the specific heat C_p or $\partial\eta/\partial s$ is known with any accuracy. The specific heat can be measured directly and $\partial\eta/\partial s$ calculated from the Gibbs-Duhem relation if μ_ω is known. The entropy at high pressure can be calculated from (5).

The specific heat C_p of sea water was first measured in 1889 by J. Thoulet and A. Chevallier using water samples taken from the English Channel. These samples were diluted by adding distilled water or concentrated by evaporation and a series of determinations of specific heat were made at a temperature of 17.5°C. Their measurements, omitting interpolated values, are reproduced in Table 2.

TABLE 2

SPECIFIC HEAT OF SEA WATER AT 17.5°C
(From J. Thoulet and A. Chevallier, 1889)

Density gm/cm^3	Ratio of Specific Heat of Sea Water and Fresh Water	Salinity (Computed from Density) %
1.0043	.980	5.6
1.0073	.968	9.5
1.0153	.951	20.0
1.0176	.949	23.0
1.0232	.939	30.4
1.0235	.938	30.8
1.0290	.927	38.0
1.0357	.921	
1.0463	.903	

The specific heat of sea water is lower than that of fresh water, being about 0.93 to 0.94 $cal.gm^{-1}deg^{-1}$ at the surface of the ocean and decreasing to about 0.88 $cal.gm^{-1}deg^{-1}$ at great depths.

A complete determination of the specific heat has been undertaken at the National Institute of Oceanography in England in cooperation with

the National Physical Laboratory. The work is being done under the direction of Dr. R. A. Cox of the Institute.

EQUATION OF STATE

Considerable effort has gone into determining the dependence of specific volume of sea water on temperature, salinity and pressure. Probably the greatest incentive for this work came from the development of the theory of geostrophic currents.

Present-day tables of sigma-t, specific volume, density, coefficient of thermal expansion, etc., are based on Knudsen's (1901) determination of density at atmospheric pressure and Ekman's (1908) determination of mean compressibility. Their measurements were reduced to a set of empirical equations containing about 40 constants.

The accuracy of the density determinations at atmospheric pressure is quite high, possibly better than one part in 10^5. The measurement of compressibility is not as accurate. The corresponding accuracy in density under pressure is about one part in 10^4.

The main features of the variation of specific volume with temperature, salinity and pressure are shown in Table 3 in terms of the first and second order derivatives. The derivatives do not change sign in the range of variables found in the ocean, with the exception of the temperature derivative at low salinity.

PARTIAL CHEMICAL POTENTIALS

The partial chemical potential is of primary interest in phase transitions occurring through freezing or evaporation, and the effect of salinity on the vapor pressure and freezing point.

Equilibrium between sea water and water vapor is achieved when the partial chemical potential μ_ω equals the chemical potential of water vapor μ_v at constant temperature and geopotential. Thus, comparing equilibria at slightly different salinities, the equation

$$\left(\frac{\partial \mu_\omega}{\partial s}\right)_{T,\Phi} = \left(\frac{\partial \mu_v}{\partial p}\right)_{T,\Phi}\left(\frac{\partial p}{\partial s}\right)_{T,\Phi} = \alpha_v\left(\frac{\partial p}{\partial s}\right)_{T,\Phi}$$

TABLE 3

SPECIFIC VOLUME AND ITS DERIVATIVES WITH RESPECT TO TEMPERATURE, SALINITY AND PRESSURE AT 0°C, 35% AND ATMOSPHERIC PRESSURE
(Pressure in Decibars)

α	$= 0.972643$	$\left(\frac{\partial^2\alpha}{\partial T^2}\right)_{p,s}$	$= +13.2 \times 10^{-6}$
$\left(\frac{\partial\alpha}{\partial T}\right)_{p,s}$	$= +50.1 \times 10^{-6}$	$\left(\frac{\partial^2\alpha}{\partial s^2}\right)_{T,p}$	$= +0.76 \times 10^{-6}$
$\frac{1}{\alpha}\left(\frac{\partial\alpha}{\partial T}\right)_{p,s}$	$= +51.5 \times 10^{-6}$	$\left(\frac{\partial^2\alpha}{\partial p^2}\right)_{T,s}$	$= +15.3 \times 10^{-12}$
$\left(\frac{\partial\alpha}{\partial s}\right)_{T,p}$	$= -762.5 \times 10^{-6}$	$\left(\frac{\partial^2\alpha}{\partial T\partial s}\right)_{p}$	$= +2.8 \times 10^{-6}$
$\left(\frac{\partial\alpha}{\partial p}\right)_{T,s}$	$= -45.3 \times 10^{-7}$	$\left(\frac{\partial^2\alpha}{\partial T\partial p}\right)_{s}$	$= +26.4 \times 10^{-11}$
$-\frac{1}{\alpha}\left(\frac{\partial\alpha}{\partial p}\right)_{T,s}$	$= +46.6 \times 10^{-7}$	$\left(\frac{\partial^2\alpha}{\partial s\partial p}\right)_{T}$	$= +14.8 \times 10^{-11}$

must hold, where α_v is the specific volume of water vapor.

From (4)

$$\left(\frac{\partial\mu_\omega}{\partial s}\right)_{T,\Phi} = \alpha\left(\frac{\partial p}{\partial s}\right)_{T,\Phi} - s\left[\left(\frac{\partial\mu}{\partial s}\right)_{T,p} + \left(\frac{\partial\mu}{\partial p}\right)_{T,s}\left(\frac{\partial p}{\partial s}\right)_{T,\Phi}\right]$$

$$= -s\left(\frac{\partial\mu}{\partial s}\right)_{T,p} + \left[\alpha - s\left(\frac{\partial\alpha}{\partial s}\right)_{T,p}\right]\left(\frac{\partial p}{\partial s}\right)_{T,\Phi}$$

so that

$$-s\left(\frac{\partial\mu}{\partial s}\right)_{T,p} = (\alpha_v - \alpha_\omega)\left(\frac{\partial p}{\partial s}\right)_{T,\Phi}$$

where

$$\alpha_\omega = \left(\frac{\partial \mu_\omega}{\partial p}\right)_{T,s} = \alpha - s\left(\frac{\partial \alpha}{\partial s}\right)_{T,p}$$

is the partial specific volume of water. A similar development considering sea water in equilibrium with ice yields

$$-s\left(\frac{\partial \mu}{\partial s}\right)_{T,p} = (\eta_\omega - \eta_{ice})\left(\frac{\partial T}{\partial s}\right)_{p,\Phi} \tag{12}$$

where

$$T(\eta_\omega - \eta_{ice}) = L$$

is the heat of fusion of ice.

The change of vapor pressure is related to the change of freezing point with salinity by the equation

$$\left(\frac{\partial p}{\partial s}\right)_{T,\Phi} = \frac{L}{T(\alpha_v - \alpha_\omega)}\left(\frac{\partial T}{\partial s}\right)_{p,\Phi} \tag{13}$$

or using the perfect gas approximation for water vapor and neglecting α_ω,

$$\frac{1}{p}\left(\frac{\partial p}{\partial s}\right)_{T,\Phi} = \frac{LM_\omega}{RT^2}\left(\frac{\partial T}{\partial s}\right)_{p,\Phi}$$

where M_ω is the molecular weight of water.

The factor LM_ω/RT^2 for S = 35‰, computed from the values

$$L = 79.69 \text{ cal./gm.}$$

$$M_\omega = 18.015 \text{ gms.}$$

$$R = 1.987 \text{ cal. mole}^{-1}\text{deg}^{-1}$$

$$T = 271.3^\circ\text{C}$$

is 0.98×10^{-2} deg^{-1}. This may be compared with the value 1.00×10^{-2}

deg^{-1} calculated from data given in The Oceans (p. 66-67), and 0.99 x $10^{-2} deg^{-1}$ from measurements of vapor-pressure lowering (Arons and Kientzler, 1954). The agreement is better than is warranted by the data.

Osmotic pressure π is related to the freezing point depression through the relation

$$\alpha_\omega \left(\frac{\partial \pi}{\partial s}\right)_{T,\Phi} = s \left(\frac{\partial \mu}{\partial s}\right)_{T,p} = -\frac{L}{T}\left(\frac{\partial T}{\partial s}\right)_{p,\Phi}$$

or

$$\left(\frac{\partial \pi}{\partial s}\right)_{T,\Phi} = -\frac{L}{\alpha_\omega T}\left(\frac{\partial T}{\partial s}\right)_{p,\Phi} \qquad (14)$$

where

$$L/\alpha_\omega T \sim 12.1 \text{ atm./}^\circ\text{C}.$$

APPLICATION OF THERMODYNAMIC CONCEPTS IN OCEANOGRAPHY

Adiabatic Temperature Gradient

In 1857 Sir William Thomson (later Lord Kelvin) derived an expression relating the temperature change with pressure in an adiabatic process. His formula may be expressed as

$$\Gamma = \left(\frac{\partial T}{\partial p}\right)_{\eta,s} = -\frac{(\partial \eta/\partial p)_{T,s}}{(\partial \eta/\partial T)_{p,s}} = \frac{T}{C_p}\left(\frac{\partial \alpha}{\partial T}\right)_{p,s} \qquad (15)$$

Adiabatic temperature changes in water were demonstrated in 1869 in a pressure tank (see C. W. Thomson "The Depths of the Sea," p. 297) using a fully protected thermometer designed by Sir William Thomson. However, as both the coefficient of thermal expansion and the specific heat were then unknown no quantitative comparisons were made. The temperature changes observed were of the order of 0.2°C for a change of roughly 500 atmospheres and the effect was considered too trivial to consider as a source of error in temperature measurements.

The first practical application of adiabatic changes appears to have been to correct temperatures obtained with insulated water bottles. Nansen pointed out that such corrections should be made, and calculated a table for estimating the temperature difference. Ekman (1905) recalculated the table using Tait's measurements of compressibility and taking into account variation of thermal expansion and specific heat with pressure which Nansen had neglected. Later, after he had measured the compressibility himself, Ekman (1914) published a set of tables for computing the adiabatic gradient in the ocean.

Interest in the adiabatic temperature gradient in the ocean was developed when it was found that the temperature in deep basins and trenches increased towards the bottom. The increase is usually observed only at depths greater than 3000 meters in the open ocean but may extend from shallower depths in the more enclosed basins.

Schott (1914) analyzed the temperature increase in the Mindanao Trench and came to the conclusion that the adiabatic gradient was exceeded and the deep water was unstable. However, more recent determinations show that the gradient is equal to or slightly less than the adiabatic gradient (see Figure 1).

The variation of the adiabatic gradient with temperature, salinity and pressure is shown in Figure 2.

Sound Velocity

The adiabatic compressibility is given by

$$\left(\frac{\partial \alpha}{\partial p}\right)_{\eta,s} = \gamma \left(\frac{\partial \alpha}{\partial p}\right)_{T,s} = \left(\frac{\partial \alpha}{\partial p}\right)_{T,s} + \Gamma \left(\frac{\partial \alpha}{\partial T}\right)_{p,s} \qquad (16)$$

where γ is the ratio of specific heats at constant pressure and at constant volume. Substituting (16) into the equation for sound velocity yields

$$V^2 = \left(\frac{\partial p}{\partial \rho}\right)_{\eta,s} = -\frac{\alpha^2}{\left(\frac{\partial \alpha}{\partial p}\right)_{T,s} + \Gamma \left(\frac{\partial \alpha}{\partial T}\right)_{p,s}} \qquad (17)$$

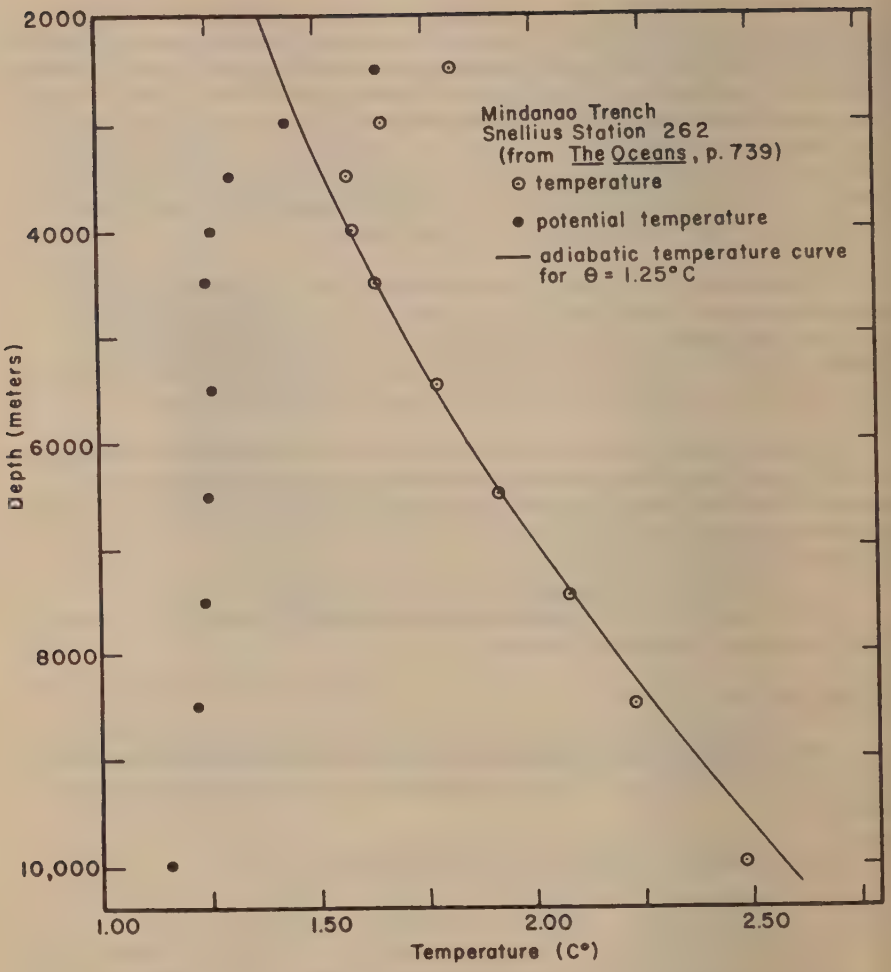
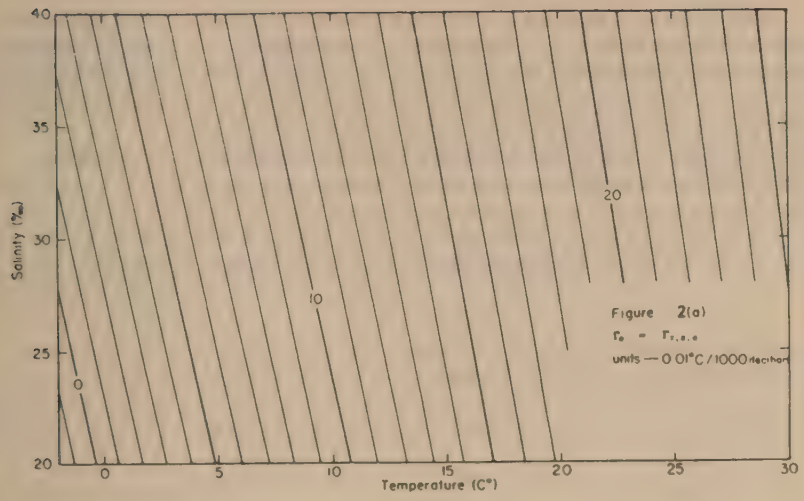


Figure 1. Actual and potential temperature in the deep water of the Mindanao Trench. The curve indicates the adiabatic change of temperature with pressure for a potential temperature of 1.25°C.



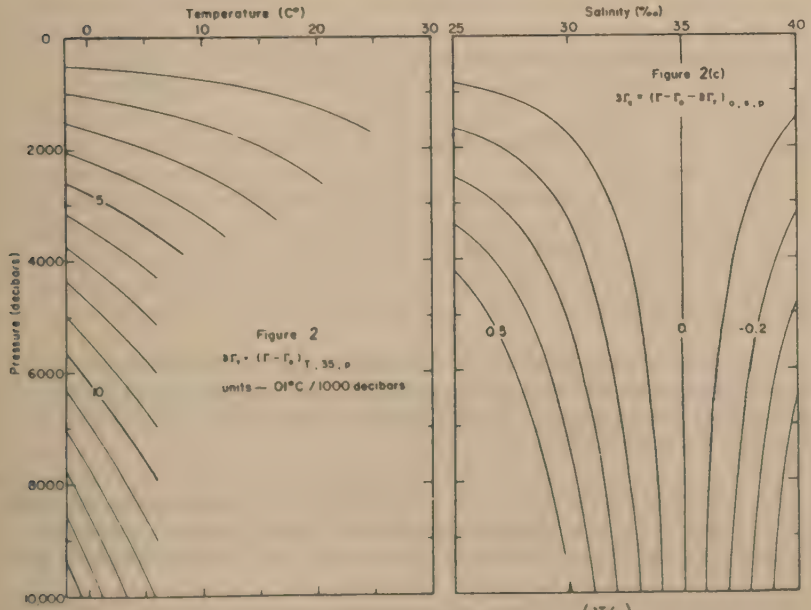


Figure 2 Variation of adiabatic temperature gradient, $\Gamma = \left(\partial T/\partial p\right)_{S,\theta}$, with temperature, salinity and pressure. The variations are given in terms of Γ_0, $\delta\Gamma_T$ and $\delta\Gamma_S$, where $\Gamma = \Gamma_0 + \delta\Gamma_T + \delta\Gamma_S$

The tables of Kuwahara (1939) are computed from (17). He tabulated sound velocity to 0.1 meters/sec., a precision of 1 part in 15,000, approximately. However, his method of computing the sound velocities yields a precision of ± 0.2 m/sec. The accuracy of the sound velocity computed by this method is considerably less (Beyer, 1954). To achieve an accuracy of ± 0.1 m/sec. in sound velocity the quantities entering into (17) must be known with the following accuracies:

	Minimum Required Accuracy	Estimated Present Accuracy
α	1/15,000	1/100,000
$(\partial\alpha/\partial p)_{T,s}$	1/6,000	1/250
$(\partial\alpha/\partial T)_{p,s}$	1/200	1/40
Γ	1/100	1/20
C_p	1/100	1/50

To achieve the same accuracy in sound velocity computations using field data, the minimum accuracy of the field data must be

Temperature	± 0.02°C
Salinity	± 0.08‰
Pressure	± 5 db.

Potential Temperature

The potential temperature is defined as the temperature of a mass of water raised adiabatically to the surface. The concept was first introduced into oceanography by Helland-Hansen (1912), although it was used earlier by meteorologists.

The potential temperature is computed by integrating the adiabatic gradient using the temperature in situ as the initial value. Tables for converting to potential temperature were given by Helland-Hansen (1930).

The primary use of potential temperatures has been to trace water masses in the deeper parts of the ocean. All of the water in a particular

region may have a common origin which is obscured by temperature differences induced by changes of pressure. Reduction to potential temperatures eliminates the effect of pressure and allows direct comparison of water masses lying at different depths in the ocean.

The classical example of the use of potential temperatures is the analysis by Van Riel of the renewal of deep water in the basins of the East Indian Archipelago. He found that the potential temperature and salinity of each basin was determined primarily by the depth of the connecting sills and could be used to trace the movement of water from basin to basin.

Potential temperatures are sometimes used to determine the stability, but this can lead to error unless the salinity is constant. A comparison of potential and actual temperatures for a deep-ocean station is made in Table 4 and in Figure 1.

Potential Density

The potential density is the density of a water mass at its potential temperature. The potential density is seldom computed by oceanographers but is of theoretical interest in the method of isentropic analysis.

Isentropic analysis was developed first by meteorologists to study flow patterns in the atmosphere. It consisted of plotting the topography of a surface of constant entropy and the contours of temperature or water vapor on the surface. The main properties of the surface are that it is invariant under adiabatic deformations and that steady-state flow will occur along the surface insofar as conditions are adiabatic and not affected by mixing. Moreover, if mixing does occur, it is more likely to be with elements of the same density in the surface.

In the atmosphere the isentropic surface is also a surface of constant potential temperature and potential density. In the ocean, the entropy is a function of salinity as well as temperature so that an isentropic surface does not coincide with the surface of constant potential temperature or of potential density. Consequently, a surface of constant potential density does not remain invariant under adiabatic deformations. However, the deviations are relatively small in the upper layers of the ocean and in practice are neglected. Moreover, because the potential density is nearly equal to σ_t, which is computed routinely for oceanographic observations, it is usual to assume that the σ_t surface, or the equivalent thermosteric anomaly surface (Montgomery and Wooster,

TABLE 4

A COMPARISON OF TEMPERATURE, POTENTIAL TEMPERATURE, POTENTIAL DENSITY AND DENSITY IN SITU FOR A DEEP OCEANOGRAPHIC STATION

Depth Meters	Temp. (t) °C	Potential Temp. (θ) °C	Salinity ‰	σ_t	σ_θ	σ_{stp}	Pressure Decibars
0	7.47	7.47	32.47	25.39	25.39	25.39	0.0
50	7.17	7.16	32.50	25.45	25.45	25.68	50.3
100	7.02	7.01	33.26	26.07	26.07	26.53	100.6
200	6.41	6.39	33.94	26.68	26.69	27.61	201.4
500	5.03	4.99	34.13	27.00	27.01	29.34	504.1
1000	3.19	3.12	34.39	27.40	27.41	32.10	1009.6
1500	2.36	2.26	34.52	27.58	27.59	34.62	1516.5
2000	1.93	1.79	34.61	27.69	27.70	37.05	2024.6
2500	1.82	1.65	34.64	27.72	27.73	39.37	2533.8
3000	1.66	1.44	34.66	27.75	27.76	41.68	3044.2
3500	1.58	1.31	34.67	27.76	27.78	43.95	3555.7
4000	1.59	1.26	34.67	27.76	27.78	46.18	4068.3
4500	1.64	1.25	34.67	27.76	27.78	48.38	4582.0
5500	1.78	1.26	34.67	27.75	27.78	52.71	5612.6
6500	1.92	1.25	34.67	27.74	27.78	56.96	6647.3
7500	2.08	1.24	34.67	27.72	27.79	61.13	7686.2
8500	2.23	1.22	34.67	27.71	27.79	65.23	8729.2
10000	2.48	1.16	34.67	27.69	27.79	71.24	10301.1

1954), fulfills with sufficient accuracy the role of potential density. For a more complete exposition of the method of isentropic analysis, the reader is referred to a paper by Montgomery (1938).

A comparison of σ_t, potential density σ_θ, and density in situ $\sigma_{T,s,p}$ is made in Table 4. In this particular example, σ_θ can be taken equal to σ_t, with negligible error, to 4000 meters. However, the approximation should not be used routinely beyond the upper 1000 meters without verification.

Potential Energy

Although potential energy is considered primarily in the study of the dynamics of the ocean system, it is discussed here because of its close relation to the thermodynamical variables.

The potential energy x of a mass of water is given, apart from an arbitrary constant, by

$$x = \int p\Phi dz = \int \Phi dm \tag{18}$$

where z is the vertical coordinate. Changes of potential energy are produced by movement of mass relative to the geopotential field. These changes can be related to changes of thermodynamic variables through the hydrostatic equation.

The total enthalpy H of a column of sea water of total mass M is given by

$$H = \int_0^M h dm$$

$$= U + \int_0^M p\alpha dm \tag{19}$$

where U is the total internal energy.

Integrating (19) by parts yields

$$H = U + (p\int\alpha dm)_o^M - \int_o^M \left(\frac{dp}{dm}\int\alpha dm\right) dm ,$$

which can be transformed, using the hydrostatic equation ($dp/dm = -g$) and $z = \int\alpha dm$, to

$$H = U + (pz)_o^D + \int_o^M gzdm .$$

If variations of gravity g with height are neglected the expression for total enthalpy reduces to

$$H = U + p_aD + \int_o^M \Phi dm - M\Phi_o$$

$$= U + x + p_aD - M\Phi_o \qquad (20)$$

where p_a is atmospheric pressure, D total depth, and Φ_o the geopotential at the bottom of the column ($z = 0$).

Changes of total enthalpy ΔH in which the total mass M is not changed are related by

$$\Delta H = \Delta U + \Delta x , \qquad (21)$$

the contribution of term p_aD being negligible.

Equation (21) can be broken down further for changes involving only a portion of the column. If this portion is called the internal system (subscript "i") and the rest of the column the external system (subscript "e"), the changes are

$$\Delta H_e = \Delta U_e = 0$$

$$\Delta H_i = \Delta U_i + \Delta x_i + \Delta x_e . \qquad (22)$$

Thus

$$\Delta x = \Delta x_i + \Delta x_e = \Delta H_i - \Delta U_i = \int \Delta p\alpha dm \qquad (23)$$

the integral being evaluated over the internal system only.

The relative magnitudes of Δx_i and Δx_e depend on the details of the change in the internal system. For example if two infinitesimal masses δm are interchanged between two levels in the column, the potential energy change of the internal system is zero, while that of the external sytem in which the initial and final pressure, temperature, and salinity are the same is not zero.

If the interchange occurs without transfer of heat or salt (i.e., isentropically) it can be shown that the change of any thermodynamical property φ per unit mass for a unit vertical separation is proportional to

$$-\frac{\partial p}{\partial z}\left\{\frac{d}{dz}\left(\frac{\partial \varphi}{\partial p}\right)_{\eta,s} - \left[\frac{\partial}{\partial z}\left(\frac{\partial \varphi}{\partial p}\right)_{\eta,s}\right]_{\eta,s}\right\}$$

$$= \rho g\left\{\left[\frac{\partial}{\partial T}\left(\frac{\partial \varphi}{\partial p}\right)_{\eta,s}\right]_{p,s}\left(\frac{dT}{dz} + \Gamma'\right) + \left[\frac{\partial}{\partial s}\left(\frac{\partial \varphi}{\partial p}\right)_{\eta,s}\right]_{T,p}\right\}\frac{ds}{dz} \qquad (24)$$

where

$$\Gamma' = -(\partial T/\partial z)_{\eta,s} = \rho g \Gamma .$$

Thus, substituting $\varphi = h - u = p\alpha$ yields

$$\Delta x_e \sim \rho g\left\{\left[\frac{\partial}{\partial T}\left(\alpha + \frac{p}{\gamma}\frac{\partial \alpha}{\partial p}\right)\right]\left(\frac{dT}{dz} + \Gamma'\right) + \left[\frac{\partial}{\partial s}\left(\alpha + \frac{p}{\gamma}\frac{\partial \alpha}{\partial p}\right)\right]\frac{ds}{dz}\right\} = E_x \qquad (25)$$

for the potential energy change of the external system.

The change of total energy ΔK of the system is equal to the sum of changes of internal and potential energies, which by (21) is equal to the enthalpy change. Substituting $\varphi = h$ into (24) yields

$$\Delta K = \Delta H \quad \rho g\left[\frac{\partial \alpha}{\partial T}\left(\frac{dT}{dz} + \Gamma'\right) + \frac{\partial \alpha}{\partial s}\frac{ds}{dz}\right] = E_H \qquad (26)$$

Stability

If ΔK is positive work must be done on the column to interchange the water masses, and the stratification within the column is

said to be stable. The stability is indicated quantitatively by the parameter $E_{H,\eta}$.

The stability parameter was first introduced by Hesselberg and Sverdrup (1914) who defined it in terms of the buoyant force acting on a unit mass of water displaced vertically from its initial surroundings. Their definition is equivalent to (26).

In general, the stability $E_{H,\eta}$ is positive, ranging from 10×10^{-8} ergs per gram per cm.^{2} in the deep water to perhaps $500{,}000 \times 10^{-8}$ ergs per gram per cm.^{2} or higher in coastal inlets where sharp density gradients are formed by fresh-water inflow. Unstable conditions are occasionally encountered in the open ocean but are more frequent in coastal areas subject to tidal action. The parameter reaches high values in the upper layers and decreases with depth. Often one or two subsurface maxima are present. These are probably caused by differential or opposing movement of the deeper waters. The stability parameter enters also into the study of internal waves.

EFFECT OF MIXING ON POTENTIAL ENERGY

If the elements of water are allowed to mix with a large amount of surrounding water, after the interchange the external potential energy change is modified to

$$\Delta x_e \sim E_{x\eta} - p\left[\frac{\partial^2\alpha}{\partial T^2}\left(\frac{dT}{dz}+\Gamma'\right)^2 + 2\,\frac{\partial^2\alpha}{\partial T\partial s}\left(\frac{dT}{dz}+\Gamma'\right)\frac{ds}{dz} + \frac{\partial^2\alpha}{\partial s^2}\left(\frac{ds}{dz}\right)^2\right] = E_x \qquad \ldots(27)$$

$$\simeq -\left\{\left[\frac{d}{dz}\left(p\,\frac{\partial\alpha}{\partial T}\right)\right]\left(\frac{dT}{dz}+\Gamma'\right) + \left[\frac{d}{dz}\left(p\,\frac{\partial\alpha}{\partial s}\right)\right]\frac{ds}{dz}\right\}$$

The additional term in Δx_e is due to the change of volume on mixing.

The parameter E_x contains quadratic terms in the vertical gradients of temperature and salinity. It becomes negative if the gradients are sufficiently large. In other words, the potential energy can be decreased by nonisentropic or irreversible changes if the gradients are sufficiently large. Preliminary studies of vertical stratification in the ocean have indicated that such gradients are reached in some regions of

the ocean but are not exceeded. However, the exact significance of the parameter E_x is obscure at present and it is not clear that changes of potential energy necessarily influence such irreversible changes. The magnitudes of the parameters $E_{x\eta}$, $E_{H,\eta}$ and E_x are compared in Figure 3.

APPLICATIONS OF OTHER THERMODYNAMICAL PROPERTIES OF SEA WATER

Freezing Point

The temperature at which ice first begins to form in sea water has been determined by Knudsen (1903) and Miyake (1939). The freezing

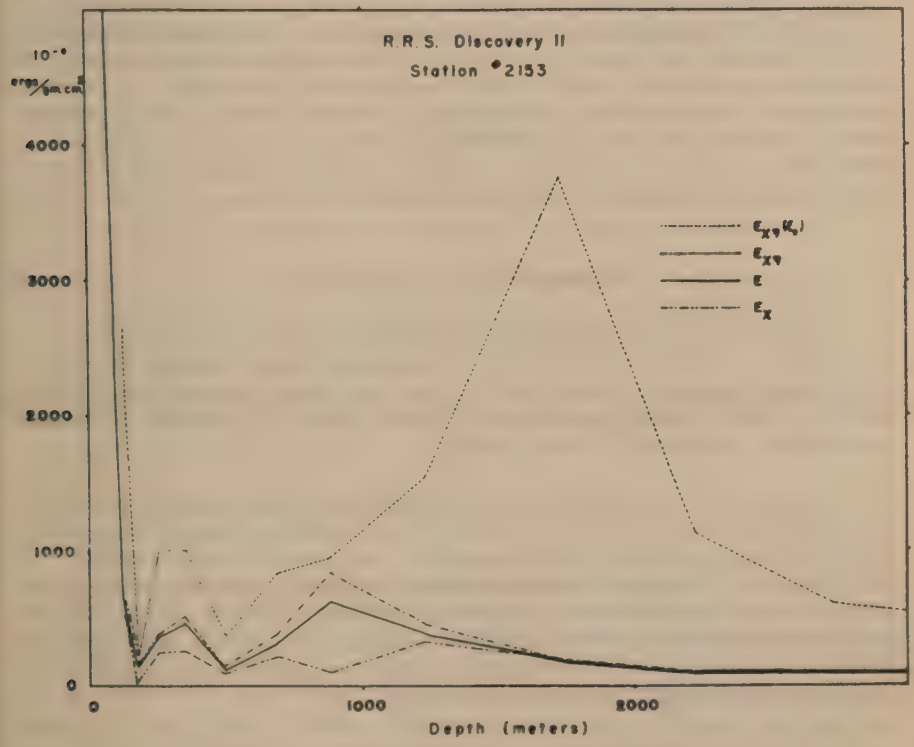


Fig 3. Variation with depth of stability E, potential energy E_x and $E_{x\eta}$. The parameter $E_{x\eta}$ is also shown at the critical gradient ξ_z at which E_x is zero.

process is of considerable importance in the formation of deep water in the Antarctic, in that it increases the salinity by removing water. The effect is cumulative in the westward current close to the Antarctic continent and sufficiently high densities are reached in the Weddell Sea to allow the surface waters to sink beneath the warmer intermediate water.

Vapor-Pressure Lowering

Saturation vapor pressure over salt water is about 2% lower than that of fresh water. Although the difference is small it is taken into account in studies of evaporation, energy transfer across the ocean surface, formation of sea fogs and the study of salt nuclei formed in the air.

Osmotic Pressure

Osmotic pressure is of primary importance in biological studies. Marine animals and organisms must excrete wastes and maintain a balance of body fluids under conditions of varying salinities, i.e., osmotic pressures. Some animals can tolerate a greater range of salinities than others. Salmon and eels, for example, spend part of their life cycle in fresh water and part in salt water. They have developed complex mechanisms for the exchange processes in these two media.

SUMMARY OF DISCUSSION

Dr. Goldberg questioned the definition of the effective chemical potential μ. It is defined in terms of energy per gram rather than the more usual energy per mole unit in order to bring in salinity as a state variable. Dr. Goldberg pointed out that the chemical potential might be expressible in terms of conductivity.

Dr. Holser stated that we need to measure only specific volume and entropy or heat capacity to determine the thermodynamic properties. The remaining quantities can be derived. Dr. Eckart elaborated this point further. He stated that all thermodynamic properties can be derived from measurements of specific heat as a function of salinity and temperature at atmospheric pressure; specific volume as a function of salinity, temperature and pressure; and $\partial\mu/\partial s$ as a function of salinity at one temperature and atmospheric pressure.

In answer to a question by Dr. Paquette, it was stated that the allowable errors in the calculation of sound velocity are individual errors and were not calculated to occur simultaneously.

Dr. Carritt questioned the existence of a unique relationship among density, salinity and chlorinity. The calculation of first and second derivatives of density is therefore a highly questionable undertaking.

Dr. Koczy pointed out that Wus's study of the movement of Antarctic bottom water through the Romanche Deep would have been a better choice of the classical example of the use of potential temperature analysis.

Dr. Eckart commented that the need for knowledge of sea-water properties often comes from unexpected quarters. He recently learned that adiabatic heating is of concern to microbiologists conducting experiments on bacteria from deep trenches.

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ASSESSMENT OF THE ACCURACY AND PRECISION OF DATA

by

John Strickland

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ASSESSMENT OF THE ACCURACY AND PRECISION OF DATA*

*John Strickland***

It is quite important that we give a little consideration to what we mean by the accuracy and precision of our physical or chemical oceanographic data and try and decide how we should report this accuracy and precision.

If we say, for example, that the chlorinity of a sample is 19.024‰ and "we must know this result to better than 0.01‰," then really we are talking nonsense, although we all make such statements verbally and, what is worse, we sometimes make them in print. Of course what we mean to say is that there is a certain probability that the true chlorinity lies within the range 19.014 to 19.034‰ and that we must be sure that this is a very high probability.

I would suggest, therefore, that we do at least three things before we make an oceanographic observation of any chemical or physical property of sea water.

1. Firstly we must assure ourselves that no systematic errors occur in our results due to the method we use or that, if errors cannot be eliminated, then they are estimated as precisely as possible and stated. It is this type of systematic error which affects what I term the accuracy of a result.

It is often very difficult or impossible to eliminate or estimate all errors, as anyone who has designed a really new piece of apparatus for making a precise measurement is fully aware. This difficulty underlies much of the work which is being described at this meeting.

2. However, in many cases, if we have well-designed and tested methods and equipment, used by competent people, the accuracy of observations may be quite high but we are still left with more or less random errors which govern what I prefer to term the precision of a measurement. We should therefore next assure ourselves that these random errors obey a Gaussian distribution and then we should find out the standard deviation (σ) characterizing this distribution. In practice it

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is often not very difficult to do this, even if there is some skewness to the distribution, and a rough estimate of o (which is greatly to be preferred to no knowledge of σ at all) can be obtained from as little as 20 or 30 duplicate measurements carried out whilst using the method concerned.

A few words of caution are necessary. If the property being measured varies greatly in magnitude, for example the silicate content of sea water, then o should be estimated for at least two or three levels, as the coefficient of variation is rarely constant, certainly not in much chemical work. We must also be honest and realistic and determine the o values for the conditions under which the method is to be used. There is no excuse for taking the standard deviation obtained in the research laboratory when the method was being worked out and using this standard deviation to analyze results obtained on board ship in a rough sea by technicians not too well trained in the art. Finally we must be clear as to what precision we wish to measure. For much chemical and physical work I suggest that this precision be that of the method, as such, when used with a single homogeneous sample of sea water. The over-all precision of a replicate determination in nature may well be much worse. Apart from sampling errors, we are not sure, especially in the upper 500 meters, whether or not the properties of the medium are sufficiently constant in between casts for no changes to occur. Any such changes will be more or less a characteristic of the location concerned and could often be made the subject of a separate study. It seems unwise to confuse them with the inherent accuracy and precision of a method.

3. Thirdly, even when the accuracy of a method is known and an approximate standard deviation has been ascertained, there is still a decision to be made. We must decide with what degree of confidence or probability we wish to know the results. The range each side of the experimental value in which the true result may lie will depend upon the degree of probability that we consider acceptable. If we want to be very certain that a given range of values includes the true result then this range will have to be larger than if a less stringent criterion is satisfactory. In other words we need to decide what multiple of σ is to be taken, a multiple that depends on the confidence limits chosen.

In most industrial measurements the 95 per cent confidence limit (19 to 1 odds in favor) is considered adequate and in my opinion this limit is also satisfactory for most physical, chemical and perhaps even biological oceanography. However, the precision criterion employed should depend entirely on the use to which the data is being put and it is

really up to the author of any report to decide what he considers an appropriate multiple of σ. I suggest that it is better that such a multiple be used by an author rather than the precision be expressed simply as $\pm\sigma$, as is sometimes done in the literature. Such a practice actually indicates quite a low precision criterion and yet may perhaps imply otherwise to an unwary reader.

Nothing that I have said here is anything but commonplace. However, much of the data I see reported in the oceanographic literature is still presented with no meaningful statement of error and precision and it may not be out of place in a meeting concerned with the physical and chemical properties of sea water to decide on how best to report these properties, especially now that increasing concern is being expressed as to their precise significance.

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I. Canadian Research Report, 1958

A. SUBAREAS 2 AND 3 — By W. TEMPLEMAN[1]

The Fisheries Research Board of Canada Biological Station at St. John's has carried out in 1958 researches in Subareas 2 and 3 on cod, haddock, redfish, and American plaice. Hydrographic sections have been taken over the area from southern Labrador to the southern Grand Bank during the period July 27 to August 26.

Cod, *Gadus callarias* L. Age determinations were made from otoliths of 800 cod taken in the Labrador area (Subarea 2) in 1950 and 1951. About one quarter of the fish were from the inshore area and the remainder were taken offshore in depths of 100-200 fathoms. The age readings indicate that in the Labrador area best survival of cod from 1940 to 1947 probably occurred in 1942, 1945, and 1947. There was relatively moderate to good survival in 1944 and 1946. A fair number of old fish in the samples may be a reflection of low fishing intensity in the area at that time.

In 1958 the emphasis on better sampling of the inshore cod fishery was continued. Observations were continued throughout the fishery at Bonavista, which is one of the most important centres of the inshore fishery. With the inshore fishery in Newfoundland generally poor in 1958, only about 8,700,000 pounds of cod were landed at Bonavista, compared with 15,300,000 pounds in 1957. Of the 1958 total, 40% was landed from handlines, 26% from traps, 4% from linetrawls (longlines pulled by hand), and 30% from longlines. The 1957 landings were made up as follows: handlined, 50%; trapped, 24%; linetrawled, 2%; longlined, 24%.

To ensure a successful inshore fishery at Bonavista and on the east coast of Newfoundland, generally, it is apparently necessary for cod which come toward shore in the warmer, surface layer to be concentrated inshore and prevented, by cold water of the coastal portion of the Labrador Current, from descending too rapidly to depths beyond the range of the gears. This portion of the current exists in summer as a cold, intermediate layer below the warmer, surface water.

This cold layer, with temperatures below 0°C, was not as close to the surface in 1958 as in 1957. In addition, severe, early-summer storms mixed the inshore waters to such an extent that cod were able from early June to move much deeper than would be possible during a calm summer. The apparent lack of concentration of cod in depths within the fishable range of the usual inshore gears is reflected in the much lower catch per unit of effort of all gears generally throughout the season as compared with 1957. Also, the fishery with baited hooks was severely hampered by an extreme shortage of fresh bait.

In 1958 the landings from the longline fishery at Bonavista were 45% lower than in 1957. The longline catch per unit of effort, both offshore in the deep water and inshore in the shallower water, was much reduced as compared with 1957, and was lower than for any other year since the fishery began. It is apparent that unfavourably warm hydrographic conditions in 1958 had an adverse effect on the inshore longline fishery. In addition, the deep-water grounds are much more heavily exploited now. When the fishery began in 1952, the deep-water stocks of cod off Bonavista had not been fished previously, and until 1956 the Bonavista longliners were the only boats fishing the area. In 1956 a few large European trawlers began fishing in the area, and in 1957 and 1958 a much greater concentration of effort by a larger number of trawlers occurred. In addition, a fleet of longliners from the Faroe Islands and from Norway fished off Bonavista and in neighbouring deep-water areas in 1957 and 1958.

Length measurements have been obtained from cod landed by longliners since 1952 from the deep water below 100 fathoms off Bonavista and comparisons made of the yearly length distributions in summer. In 1952, the first year of com-

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mercial longlining, 30% by number of the cod landed were 81 cm and larger, and the average length was 76.0 cm. Each year that followed, the proportion of these larger fish in the catch was reduced and by the end of July 1958, their contribution to the catch was only 6%. The average size of the longline cod has been reduced by about 10 cm in the 1952-58 interval and the fish landed in 1957 and 1958 were mainly between 50 and 80 cm in length, with greatest numbers at 62 cm. In 1958 it is apparent that even these fish were caught by the Bonavista longliners in smaller numbers than in 1957.

Observations were conducted at Isle aux Morts on cod from the fishery on the western part of the south coast of Newfoundland. Small motor boats, motor dories, and longliners all using the same type of longlines fished 3 to 5 miles from shore in depths ranging from 50-80 fathoms. Sizes of fish caught ranged from just over 30 cm to about 130 cm, but during the fishery of March and April few cod larger than 80 cm were taken. By the middle of April there were indications of an increase in the numbers of large cod relative to the numbers of smaller ones as compared with early March, and in early July this change was obvious.

The age composition in March-April indicated a great range of ages from 4 to over 20 years of age, with fish from 5 to 11 years old making up the majority. In April the older age groups (beyond 8) were more abundant relative to the younger age groups than in March. It is apparent that in July the numbers of the older fish relative to the younger ones were even greater than in April.

The March-April samples were largely made up of fish of the stock which migrates into the Gulf in April-May, whereas the July sample would be composed of fish of the "resident" stock.

The change in size and age composition was reflected in a large decrease in the catch per unit of effort beginning in May. The reduced catch per unit of effort throughout summer was apparently because the resident stock was far less abundant than the migratory stock which mingled with it during winter and on which the success of the winter fishery depends.

Haddock, *Melanogrammus aeglefinus* (L.). In May the yearly otter-trawling surveys for haddock were carried out by the *Investigator II* on the southern half of the Grand Bank and on St. Pierre Bank. The Grand Bank survey was made nearly a month earlier in 1958 than in 1957, and bottom temperatures were on an average higher in 1958. As in 1957 the haddock had already spread northeastward from their winter quarters in the deeper water of the southwest slope to occupy much of the southern half of the bank and hence were not very abundant at any of the stations. In the area as a whole, haddock were not quite as abundant as they were in 1957. Indications are that, due to the more favourable temperature conditions in 1958, haddock spread over the bank earlier than usual. This is also reflected in the commercial landings for 1958, which decreased rapidly about a month sooner than in 1957.

The St. Pierre Bank survey for haddock was made about five weeks earlier than in 1957 and bottom temperatures over the bank were favourable for haddock. However, as in 1957, no significant quantities of any year-classes were obtained, indicating that there has been no significant survival of young haddock on this bank since the extremely abundant 1949 year-class which maintained the commercial fishery there during 1954-56.

From the catch length-frequencies and the age analysis of the samples from the Grand Bank haddock cruise in May, the most abundant group present in 1958 was the 1955 year-class with a mode at 30 cm, which will undoubtedly contribute significantly to the commercial fishery in 1959 and still more by 1960. This group accounted for more than 50% by number of the catches of the *Investigator II*. The once very abundant 1949 year-class has been relatively much reduced, accounting for less than 10% by number in the catches. The moderately abundant 1952 and 1953 year-classes with modes at 38 and 41 cm, which were important commercially in 1958, are on the decline and will be replaced in importance by the 1955 year-class. The 1956 year-class does not appear to have survived very well, and the same is true of the 1957 year-class.

In 1958 for the first half of the year the main fishery for haddock was concentrated in the southwestern Grand Bank area (Subdivision 3O) Most of the haddock landed from this fishery were from 33 to 50 cm in length with a modal group at 40 cm, made up mainly of fish from the 1952 and 1953 year-classes.

In the second half of 1958 a limited fishery for haddock occurred in the southeastern Grand Bank area. Haddock landed were from 30 to 50 cm in length with modal groups at 33 to 34 cm and at 41 cm. The 33 to 34 cm modal group represents the larger fish of the 1955 year-class which were retained, and the 41 cm group represents fish of the 1952 and 1953 year-classes.

It is obvious that for the next two or three years fish of the 1955 year-class will be important in maintaining the Grand Bank haddock fishery.

Redfish, *Sebastes marinus mentella* Travin and *Sebastes marinus marinus* (L.). Researches in 1958 indicated that the *marinus* type redfish could be more plentiful and have somewhat different habits than had previously been known for the area. The *Investigator II* in a half-hour drag east of Hamilton Inlet Bank on June 10 at 250-256 fathoms (460 m) caught 480 pounds of large *marinus* type redfish per hour's dragging and only 80 pounds of the *mentella* type. Almost all the *marinus* type females had larvae ready or nearly ready for extrusion. This capture of significant numbers of spawning *marinus* type redfish at a depth where usually only *mentella* type redfish exist is similar to that reported by Lundbeck in the German Research Report, 1957 (ICNAF Ann. Proc. Vol. 8) of spawning concentrations of *marinus* type redfish at 420-450 m off East Greenland.

In 1958 commercial redfish fishing began in Subarea 2 of ICNAF in an area off Hawke Harbour, Labrador, which was first fished by the *Investigator II* in September 1951. The part of this new commercial fishing area fished by Newfoundland trawlers is about 80 to 120 nautical miles south of the area east of Hamilton Inlet Bank which has been fished successfully for redfish by the *Investigator II* usually in August or September, from about 140 fathoms down to about 200 fathoms in each year from 1950 to 1953, and down to 300-400 fathoms in 1954, 1956, 1957, and 1958.

Redfish fishing in this new commercial area off Hawke Harbour has been very successful and has been participated in by Canadian, United States, Icelandic, German, Belgian, and Russian trawlers. The Newfoundland fishery occurred mainly between 150 and 180 fathoms (occasionally 125-200 fathoms) in late August 1958, and produced a catch of between 2 and 3 million pounds of redfish. These catches were all from Subdivision 2J, mainly between Lat. 52°45′ and 53°00′N and Long. 52°10′ and 52°40′W, and with good catches also about 40 nautical miles north of this area. The northern part of the Newfoundland fishing area was on the southeastern part of the Hamilton Inlet Bank area and the more southern fishery which was much greater was on the fringes of Hawke Channel, the below-200-fathom indentation in the continental shelf leading toward Hawke Bay.

Sampling of some of the commercial catches from the area has shown that fair numbers of *marinus* type fish occurred in the catches, and between August 25 and September 25, 1958, when 1% of a total catch of one and a half million pounds was examined, 11% by numbers were found to be *marinus* type fish. Since specimens of *marinus* were on the average more than twice as heavy as an average specimen of *mentella*, the *marinus* type was considerably more important by weight than by relative number.

The data from this sampling would indicate that *marinus* type fish are, in this area, relatively more abundant than we had formerly supposed and that on occasional trips, a very high percentage of a trawler's catch may be *marinus* type.

In a cruise of the research vessel *Marinus* from May 5-22, 1958, just east of Hermitage Bay, at the northern entrance to Connaigre Bay on the south coast of Newfoundland at 112-116 fathoms, 146 *marinus* type redfish were found in an hour's towing during which 4,500 pounds of redfish were caught. The remaining approximately 4,000 redfish were of the usual *mentella* type.

Also on the east side of Connaigre Bay just below the edge of the 100-fathom contour and at the same time of the year one dragger caught 7,000 pounds of redfish in a 2-hour drag and the captain said that over half of these (probably by weight) were of the *marinus* type. Another dragger also fishing in the same area reported over half its catch to be *marinus* type. These *marinus* type fish which are typically golden yellow in colour in this area, are on the average considerably larger than the *mentella* type, and are readily recognized by the trawler skippers.

Flemish Cap has continued to receive our attention since 1950, when large catches of redfish were obtained there by the *Investigator II*. The area has, as yet, been little exploited by Canadian vessels, although vessels of the USSR have caught many millions of pounds there since 1956.

During the fishing trials of the new research vessel of the Fisheries Research Board, the *A. T. Cameron*, the opportunity was taken to examine again the general pattern of the redfish distribution at various depths in the area on the north and northeast slope of Flemish Cap. A total of 15 sets was made in the area at depths between 112 and 410 fathoms. The *A. T. Cameron* appeared to fish well at all depths and good catches of redfish occurred to 300 fathoms. In greater depths, e.g. 400 fathoms, although redfish were scarce, the catches of other fish indicated that the trawl was still fishing efficiently.

As in previous trips to this area, *marinus* type redfish were abundant in the shallower water of 150 fathoms and in the two daytime sets when large catches were obtained, 78% and 66% (by number) of the redfish were *marinus* type. In two sets at 200 fathoms the combined data show only 1.6% of the catch to be *marinus* type fish and at 250 fathoms the number was further reduced to only 0.3%. At depths greater than 250 fathoms no *marinus* type fish were found.

In separating the *marinus* and *mentella* forms of redfish, only those which could be separated with complete confidence were typed and those in which any doubt existed were classed as "indefinite" or "doubtful" types. The indefinite-type fish occurred only in the shallow water (150 fathoms or less). This is mainly due to the fact that greater numbers of small fish occurred at these depths although the occasional larger fish which showed intermediate characters also occurred.

The largest catches occurred during the mid-day period in 150 fathoms where catches of 6,200 and 7,300 pounds per hour's dragging were obtained. These catches consisted mainly of large *marinus* type fish and this is reflected in the average weight per fish, which is, for these sets, approximately 2 pounds. At 200-300 fathoms, where very few or no *marinus* type fish were present, the catches declined to between 3,000 and 5,000 pounds per hour's dragging, but greater numbers of the smaller, deep-water, *mentella* type fish were taken. Below 300 fathoms, with the exception of a set which extended from 350-280 fathoms, catches declined and at 400 fathoms only 7 redfish were taken in a 40-minute drag.

A study was undertaken during 1958 of the differences between late-stage pre-extrusion larvae of *marinus* and *mentella* type female redfish obtained from selected areas around Newfoundland. The study has revealed a very interesting difference between the two types in so much as larvae from *mentella* type parents nearly always possessed one or more caudal melanophores, situated ventral to the vertebral column, posterior to and separated from the ventral row of body melanophores, whereas in larvae from *marinus* type parents these caudal melanophores were usually absent or if present, were in fewer numbers. The difference is a quantitative one and is useful for distinguishing populations of *marinus* and *mentella* type redfish and probably for deciding whether the so-called intermediate-type redfish are actually of *marinus* or of *mentella* type, or whether early stage larvae captured at sea are predominantly *mentella* or *marinus* type. It cannot, however, generally be used as an absolute diagnostic character in the type-identification of single redfish larvae.

American plaice, *Hippoglossoides platessoides* (Fabr.). Monthly sampling of American plaice from St. Mary's Bay has been continued. The same dominant year-classes were found as in

1957 and it appears that there is a strong survival of larvae in alternate years. Age determinations of commercial plaice from Subdivisions 3L and 3N show that plaice from the latter subdivision reach a larger size at a comparable age. From 3N the bulk of the commercial catch is of male fish aged 7 to 18 years and females of 7 to 25 years. From 3L the catch is of male fish aged 10 to 18 years and females of 10 to 24 years.

Hydrography. The customary six hydrographic sections were taken as usual in July and August. These sections extended from southern Labrador to the southern slope of the Grand Bank and across the banks and Labrador Current seaward to a depth of 500 metres at the seaward slope of the continental shelf. Once or twice each month temperatures and salinities were taken at a station 2 miles off Cape Spear, near St. John's, in 176 metres.

The hydrographic section off Seal Islands, Labrador, (Fig. 1) was taken August 4-5, 1958, and revealed that the unusually high temperatures (3.9 to 4.7°C) which were present in the deep water in 1957 had disappeared and that the temperatures of the deep water had reverted to a more normal 3.3 to 3.7°C. The inshore upper

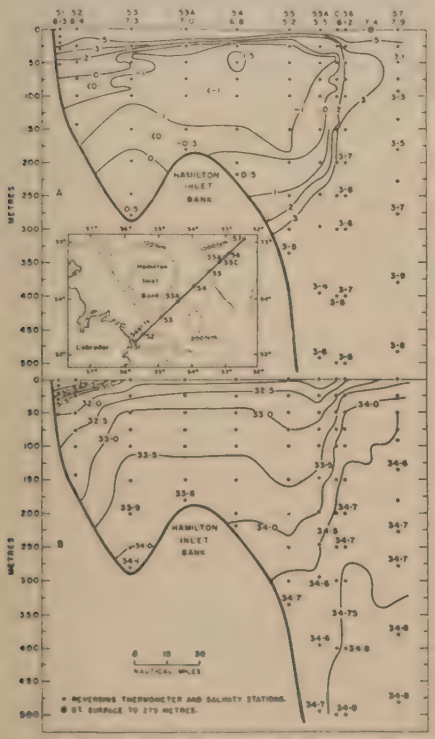

Fig. 1. Hydrographic section off Seal Islands, across Hamilton Inlet Bank, Labrador, August 4-5, 1958. A—Temperature °C; B—Salinity °/oo.

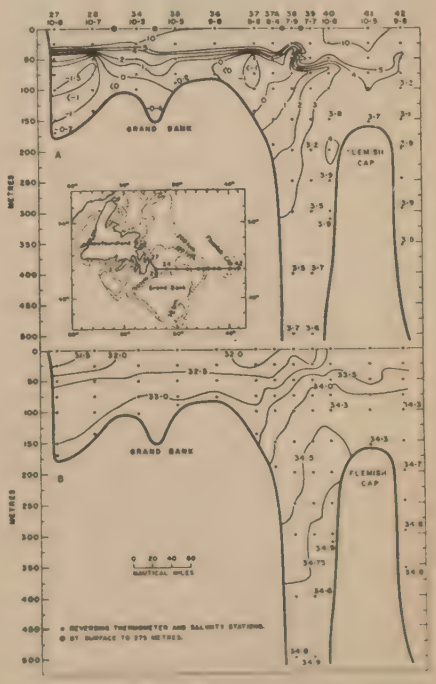

Fig. 2. Hydrographic section St. John's-Grand Bank-Flemish Cap, July 27-30, 1958. A—Temperature °C; B—Salinity °/oo.

layer of water with temperatures higher than 0°C was very much deeper in 1958 than in 1957. The salinity of the deeper water was slightly lower in 1958 than in 1957.

In the section off Bonavista, also, as in the Labrador section, temperatures in the deep water were more normal; almost a degree lower than in 1957. As in the Labrador section, the upper, surface, cold (below 0°C) layer extended deeper than usual in 1958 whereas it was shallower than usual in 1957. This coincided with a relative failure of the inshore cod fishery on the east coast of Newfoundland in 1958 compared with the much more successful fishery of 1957. A core of water with temperatures below -1.5°C was present in 1958 but not in 1957.

In the St. John's-Grand Bank-Flemish Cap section, (Fig. 2), mainly along the 47° Latitude line, the bottom temperatures of the shallower parts of the Grand Bank were slightly above 0°C in 1958 whereas in 1957 they were below 0°C. There was also considerably less water below -1°C in the shoreward portion of this section in 1958 than in 1957.

Seasonal cruises to the south and east of Newfoundland were carried out by the Atlantic Oceanographic Group, St. Andrews, N. B., in February and March and in October and November. These surveys covered a large area including the Laurentian Channel, the deep waters to the west, south, and the east of the Grand Bank, the Grand Bank, and the continental shelf east of Newfoundland as far north as the Bonavista section.

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I. Canadian Research Report, 1958

B. SUBAREAS 4 AND 5 — By W. R. MARTIN[1]

Canadian research in Subarea 4 during 1958 was carried out by the Fisheries Research Board of Canada and the Quebec Department of Fisheries. The offshore species studied were haddock, *Melanogrammus aeglefinus* (L.), cod, *Gadus callarias* L., American plaice, *Hippoglossoides platessoides* (Fabr.), redfish, *Sebastes marinus* (L.), and sea scallop, *Placopecten magellanicus* (Gmelin). The research is described by species of groundfish under the following headings: tagging, surveys, statistics and sampling, discards at sea, and gear selection. Scallop observations in Subarea 5, and hydrographic research which is pertinent to ICNAF, are briefly summarized.

Tagging. The Biological Station, St. Andrews, N. B., continued its tagging program to obtain information on populations, growth, and mortalities.

Sixteen per cent of the 1,085 haddock tagged at the mouth of the Bay of Fundy in November-December 1957 were recovered by the end of 1958. Winter recaptures of Passamaquoddy Bay haddock were taken in large numbers from the Jeffrey's Ledge and South Channel regions of Subarea 5, and in small numbers from the Digby Neck and Browns areas of Subarea 4. Summer recaptures were mainly from the tagging area in Subarea 4. Although few haddock appear to cross the deep-water Fundian Channel separating Browns and Georges Banks, haddock do move seasonally from the northern Bay of Fundy area of Subdivision 4X to the New England shore of Subdivision 5Y.

In 1957 and 1958 cod were tagged at the Magdalen Islands in order to study the relation of these cod to other populations in Subarea 4. Earlier cod taggings in the northern part of Subdivision 4T have shown few recaptures from the Magdalen Islands cod fishery. The distribution of recaptures is shown in Figure 1. Cod tagged in mid-summer 1957 were recaptured in the tagging area during the remainder of that summer, and outside the Gulf of St. Lawrence, along the 100-fathom contour, from January to April.

Fig. 1. Recaptures during 1957 and 1958 of cod tagged at the Magdalen Islands in Subdivision 4T in July 1957 and May 1958. Left, recaptures from December to May; right, recaptures from June to November.

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In the spring recaptures were reported from the Magdalens area, and in summer months these cod had spread throughout Subdivision 4T with concentrations on the northern grounds off Bonaventure and Miscou Islands. Magdalens cod move northwest during spring months and southeast in the autumn. As noted in earlier taggings of cod and haddock in Subdivision 4T, few recaptures are reported from the other side of the deep-water Laurentian Channel, but they do move seasonally between Subdivisions 4T and 4V. In 1958 about one quarter of the winter returns were from French, Portuguese, and Spanish fishermen. All other returns were reported by Canadian fishermen.

Plaice were tagged in the northern part of Subdivision 4T in June and October 1958. A high percentage (27) of the 320 plaice tagged southeast of Shippegan Gully in June were recovered by the end of 1958. Most recaptures were taken from the tagging area, but some plaice moved offshore during the summer. Two fish, recaptured in September, had moved about 100 miles north, one to the Bay of Chaleur and the other to Bonaventure Island.

Surveys. In August 1958 the Sable Island-Emerald Bank area of Subdivision 4W was surveyed with the *Harcngus*, and in May to November the northern part of Subdivision 4T was surveyed with the *J. J. Cowie* by the Biological Station, St. Andrews, N.B. The Marine Biological Station, Grand River, P.Q., extended the latter survey into the Bay of Chaleur in May to October. The survey programs are concerned with the seasonal distribution of haddock, cod, and plaice, and prediction of changes in the fishery.

The dominant species in the Subdivision 4W survey was haddock. In shoal water (10-20 fathoms) near Sable Island, large numbers of small haddock (mode 20 cm) were taken. In 20-45 fathoms the haddock were larger (mode 30 cm). At the edge of the bank, in 50-80 fathoms still larger haddock (mode 50 cm) were taken along with small haddock (mode 20 cm). These larger haddock (mode 50 cm) were more abundant on Emerald Bank (40-44 fathoms). A continuation of this program in winter and summer months will lead to prediction of the relative importance of new year-classes before they enter the fishery.

Young cod of the 1953 year-class were dominant in 1958 commercial landings from Subdivision 4T. As a result, the average size of cod in dragger landings dropped to 60 cm, the smallest mean size since 1947. Surveys in 1957 and 1958 have shown modal size groups at 22-25, 31-37, and 40-46 cm, representing 2, 3, and 4-year-old fish. The 1954 year-class appears to be above average abundance, and it is expected that it will replace that of 1953 as the dominant year-class in 1959 landings. As a result, no appreciable change from the small average size of cod landed in 1958 is anticipated in 1959.

The abundance of cod in the Bay of Chaleur increased from June to August with migration into the Bay, and decreased in October with the beginning of offshore migration. Mid-water line fishing in 1958 showed that some mature cod move off bottom in July and August. Euphausiacea appear to be more accessible to cod where they are concentrated at the thermocline.

Statistics and Sampling. Data on landings, abundance, and sizes of groundfish will be published in the "Statistical Bulletin" and the "Sampling Yearbook."

Canadian landings from Subarea 4 have increased substantially during post-war years with the adoption of more efficient fishing methods. Otter trawlers now take half the groundfish landings, including most of the redfish, flounders, and haddock, about half the cod, and a small fraction of the halibut and pollock. Cod landings continue at a high level, but with the increased landings of small-mouth species the cod catch is only about half the total groundfish catch.

With more intensive fishing, catch per unit effort and average size of most groundfish have decreased. Young, immature fish dominated haddock and cod landings in 1958. The 1952 year-class of haddock was dominant in Subdivision 4W. Various year-classes of cod, 1949 to 1955, were dominant in the landings from different populations of Subarea 4. In the Nova Scotia area, 1949 and 1952 year-classes dominated the spring catches of mature cod. Immature

cod of the 1954 and 1955 year-classes were dominant for the remainder of the year. In the Gulf of St. Lawrence, the 1953 year-class replaced that of 1950 as the dominant year-class in cod landings.

Discards. The sizes and quantities of groundfish caught and discarded at sea vary with the sizes of fish available to the gear, the selective properties of the gear, and the sizes of fish acceptable for landing. During 1958, 16 trips on commercial otter trawlers were carried out by the Biological Station, St. Andrews, N. B., to assess discards of haddock and cod.

In 1951-52 on Nova Scotia Banks there were large numbers of haddock of about 30 cm in length; the codend mesh size was $2\frac{7}{8}$ inches; and the commercial cull for gutted haddock was between 40 and 45 cm. Under such conditions, 40 to 60% by weight of the haddock caught by otter trawlers were discarded at sea.

In 1958 small haddock were still numerous; the codend mesh size had increased to more than $4\frac{1}{2}$ inches as a result of ICNAF mesh regulation; and the commercial cull was lower, 40 cm for gutted haddock and 38 cm for those landed round. It was observed, on 6 commercial trawler trips, that only 3 to 7% of haddock were discarded, by weight, on 3 gutted-haddock trips. On trips where most haddock were landed as round fish, discards were only 2 to 4% by weight. For the small portion of these trips where haddock were gutted for landing, 33 to 37% by weight were discarded at sea. It appears that the large-mesh nets are releasing virtually all haddock below commercial size. and meshes larger than $4\frac{1}{2}$ inches, manila, or the equivalent with other twines, would release some marketable haddock. The advantage of a larger mesh size would depend on growth and survival of released haddock.

Ten summer trips on commercial draggers in Subdivision 4T provided data on discards of cod. Four trips were made on draggers using small-mesh (3–$4\frac{1}{2}$ inches) and 5 trips were on draggers using large-mesh ($4\frac{1}{2}$ inches) manila codends. These draggers fished mainly on Bonaventure Island grounds. A tenth trip was made in Chaleur Bay. The average discards on the small-mesh trips were 23% by number and 9% by weight. On the large-mesh trips average discards were 12% by number and 5% by weight. These results confirm those of 1957 in showing that $4\frac{1}{2}$-inch mesh codends reduce discards by about half.

Although it is believed that a still larger mesh size would be advantageous for Subarea 4 cod, initial losses might be too high to warrant premature action by ICNAF. Fishermen's cull appears to vary with the sizes of cod available to the fishery. In 1957 New Brunswick draggers culled cod at about 50 cm; but with smaller fish in 1958 catches, the 50% cull point dropped to about 46 cm. Selection factors for manila codends have been found to be variable for cod. In some experiments the selection factor has been 3.7, and in others (see below) only 3.3. Large-mesh nets are probably more efficient, but the amount is not adequately known. Finally, the growth and survival of released cod are poorly understood. All of these variables are being investigated.

Gear Selection. Various gear-selection studies were carried out in Subarea 4 during 1958. The Biological Station, St. Andrews, N. B., investigated the selectivity of a $5\frac{3}{4}$-inch mesh manila codend for cod, a 5- to $5\frac{1}{4}$-inch manila codend for haddock, a $4\frac{5}{8}$-inch mesh nylon codend for haddock, and the effect of chafing gear, as prescribed by ICNAF, on escapement of haddock. The Quebec Experimental Fishing Station, LaTabatière, P.Q., studied the selectivity of cotton and manila codend meshes for redfish, and the selectivity of 5-inch cotton meshes in traps for cod. The Marine Biological Station at Grand River, P.Q., studied selectivity of No. 14 and No. 17 Mustad hooks in line fishing for cod.

A selection factor of 3.3 was observed for a 5- to $5\frac{1}{4}$-inch mesh codend made of double, 75-yard, 4-ply manila twine, and fished for haddock.

A piece of chafing gear, 18 meshes long, attached 4 meshes ahead of the splitting strap and along the laceage to 3 meshes from the cod-line mesh, of $4\frac{7}{8}$-inch manila, and $1\frac{1}{2}$ times the width of the codend, did not have a measurable effect on the escapement of haddock from the same codend.

A higher selection factor, 3.9, was found for a 4⅝-inch mesh codend made of double-strand, 85-yard, braided nylon twine, and fished for haddock. The experiment indicates that a 4-inch double nylon mesh is equivalent in selection to a 4½-inch double manila mesh.

In all three experiments, a No. 36 Yankee manila trawl was towed by the M.V. *Harengus* on Sable Island and Emerald Banks in August and September 1958. A cover of 1½-inch stretched-mesh Nyak was used to catch the fish which escaped through the codend meshes.

Another covered codend experiment was carried out in the summer of 1958 while fishing the M. V. *J. J. Cowie* for cod in the northern part of Subdivision 4T with a ¾ No. 35 Yankee cotton trawl. A selection factor of 3.3 was observed for a 5¾-inch mesh codend made of 75-yard, 4-ply manila twine. Higher selection factors had been observed earlier in double-manila, smaller-mesh experiments for cod.

Redfish selection experiments in Subdivision 4R gave the following selection factors: 2.4 for 3½-inch mesh and 2.8 for 4⅜-inch mesh single cotton codends; 2.2 for 3¼-inch mesh, and 2.5 for 5-inch mesh double-manila codends. These findings are consistent with earlier results for redfish.

The most frequent cotton mesh size in the back of codtraps is 4 inches. A preliminary experiment, using 3- and 5-inch meshes of single-cotton twine, gave a selection factor of 4.0. Further tests are needed.

There was little difference in the size composition of cod taken with No. 14 and No. 17 Mustad hooks on line trawl fished off Grand River, P.Q. Further work is required to compare the selectivity of these hooks with that of otter-trawl meshes.

Sea Scallop. Two sea trips to Georges Bank were made by the Biological Station, St. Andrews, N. B., on commercial scallop draggers in August and October 1958 to observe quantities and sizes of scallops discarded at sea. There were remarkable differences in the density and size distribution of scallops from bed to bed. The shucking facilities were saturated most of the time, and high catches resulted in high discards. Discards by number varied from 55 to 86%. The mean selection size varied from 95 to 100 mm shell height. Landings per boat appeared to be a measure of shucking power and sizes of scallops saved, rather than of scallop abundance. The present 3-inch ring size in scallop drags has a mean selection size of about 72 mm. The advantages and disadvantages of a larger ring size are being considered.

Hydrography. In July and August 1958 the Atlantic Oceanographic Group, St. Andrews, N. B., took part in the IGY "Deep Water Circulation" Project by occupying stations along a section between Bermuda and Baffin Bay and also stations from various points off the Canadian east coast to the IGY line. A section between Greenland and the Strait of Belle Isle was also occupied. During this cruise observations were made in Subareas 1, 2, 3, and 4; they included temperatures, salinities, dissolved oxygen, phosphate, silicate, and nitrite. Biological observations were made for the full extent of the cruise.

A survey of the central sector of the Scotian Shelf was made four times in 1958. This phase of the work is a contribution to the IGY project "Polar Front Survey — North Atlantic". The temperature and salinity distributions in the section off Halifax are given in Figure 2. In May, August, and October 1958 the intermediate temperature layer was less developed than during recent years. The bottom waters of the Scotian Gulf were colder than those observed in recent years in April, May and August. The bottom temperatures over Emerald Bank, between 6.0°C and 8.0°C, were within the limits of observations of temperature generally observed in those seasons.

Analysis of volume transport through Cabot Strait for a series of 18 seasonal sections shows, on the average, the maximum outflow in the autumn, the maximum inflow in the winter. The net transport through Cabot Strait in the deep layers, of temperature greater than 1°C, indicates that the resultant flow for all seasons is generally directed into the Gulf. The maximum net transport occurs in the autumn-winter half of the year. The maximum speed of the current within

Fig 2. Hydrographic sections off Halifax in 1968. 4-5 April, 24-25 May, 2 August and 18-19 October. **Temperature °C. Salinity °/₀₀.**

the deep layers was computed to be 10 cm per second along the Cape Breton coast and 20 cm per second along the Newfoundland coast.

Analysis of surface water temperatures along the coast shows that the main feature of temperature variations from 1957 to 1958 was an increase at all stations during the first 6 months of the year and a general decrease during the second half of the year.

As in earlier years, the Marine Biological Station, Grand River, P.Q., carried out hydrographic observations in Chaleur Bay.

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II. Top Chafing Gear Studies

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At the 1958 Annual Meeting of ICNAF the prevalent use of top chafing gear was recognized. Recommendations from this meeting assigned highest priority in chafing gear studies to covered-net selection trials with the prescribed ICNAF type top chafing gear. During September 3-6, 1958, experiments with top chafing gear were carried out in Subdivision 4W where haddock of adequate size were taken in good quantities by the M.V. *Harengus*.

These experiments show that topside chafing gear of the same mesh size as the codend, 1½ times the width of the codend and to ICNAF specifications (described below), did not reduce escapement of haddock.

Methods

Twenty-three tows, each of 45-minutes duration, were carried out with a No. 36 manila trawl (60-foot headline). A new, double-strand, manila codend of 75-yard, 4-ply, untreated twine was used. Mesh sizes of the codend are shown in the following table, as measured in series along the length of the codend with the ICNAF-type wedge gauge.

Mesh size averaged between 4 7/8 to 5 inches, with considerable variation along the length of the codend. Larger meshes, about 5 inches, were found in the aft half of the codend. Within this portion of the codend the meshes were largest, about 5 1/8 inches in the bag. Since with moderate catches the aft portion of the codend has been shown to be most effective in allowing fish to escape, the codend mesh size of the aft half, about 5 inches, has been used in calculating selection factors.

The topside chafing gear used complied closely with ICNAF specifications. A new piece of double manila netting, 18 meshes long, was attached across the codend 4 meshes ahead of the splitting strap (halving becket) attachment. This piece of netting was attached along the lace-age (selvage) to a point 3 meshes from the cod-line mesh.

Because this netting was new and not subjected to much strain while fishing, shrinkage was excessive. To counteract this, the piece of netting was stretched with the trawl winch after the first tow. Mesh size of the chafing piece became about 4 7/8 inches and inspection on deck indicated that about the 4 aftermost meshes of the codend were clear.

The top chafing netting was 1½ times the width of the codend, although the width was probably not wholly effective, since the cover over the whole was only about 1/3 wider than the codend itself. The cover used was of 1½-inch mesh, single Nyak (nylon and cotton).

Twelve successful covered-codend tows without top chafing gear were carried out in sequence (15 tows were made but the first three were discarded). Following these, the topside chafing gear was attached to the codend under the cover

Mesh size of manila codend used for chafing gear experiment

Date	Mesh size			
	No. meshes measured	Aft half (inches)	Forward half (inches)	Average (inches)
Sept. 4/58	76	5	4¾	4¾
Sept. 5/58	78	5 1/16	4¾	5
Sept. 6/58	78	5	4¾	4¾

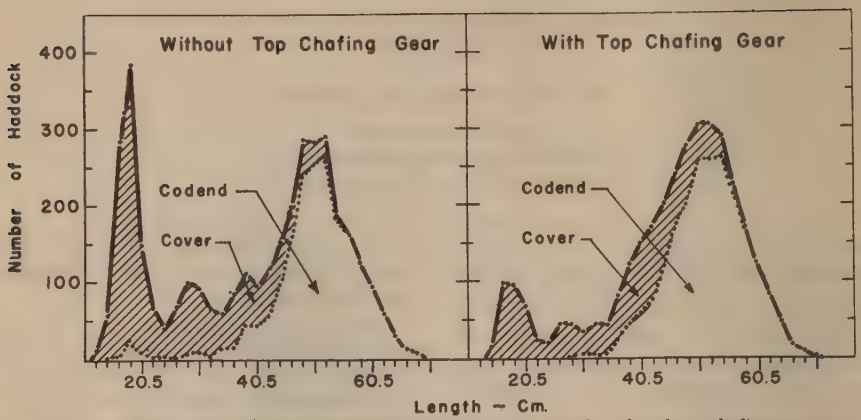

Fig. 1. Number and sizes of haddock in codend and cover with and without chafing gear.

and 8 successful tows made. The results are shown in Figure 1.

Catches of haddock averaged about 1,000 pounds per tow. Numbers of haddock within the effective selection range were similar in the two portions of the experiment. However, many more small haddock (mode about 20 cm) were taken during the trials without chafing gear. Numbers and sizes of haddock retained in the codend and escaping into the cover are shown in Figure 1 for each portion of the experiment. The resulting selection curves for the codend with and without top chafing gear are shown in Figure 2.

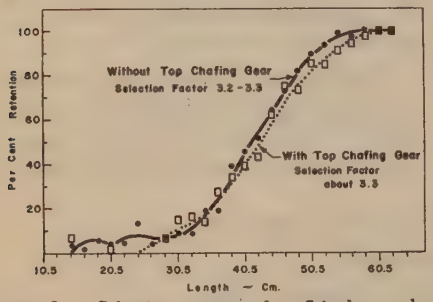

Fig. 2. Selection curves for 5-inch mesh, double-strand, manila codend with and without top chafing gear.

Both selection curves are quite similar in shape and position with a 50% retention length for the 5-inch mesh codend of about 41-42 cm. The selection factor for each is about 3.2 or 3.3.

Discussion.

With catches of the size shown, top chafing gear mounted according to ICNAF specifications had no influence on retention within the codend. Since the effective slack of the top chafing gear was less than that specified by ICNAF, the results suggest that netting less than 1½ times the width of the codend might be used.

Netting 1½ times the width of the codend is awkward and bulky on top of the codend. It would seem desirable to use a narrower top chafing gear if it does not affect escapement. It should be remembered that the 1½ times width specified by ICNAF regulation was arbitrarily chosen to provide what was believed to be ample slack. If experimentation shows that this width is more than enough, reduction in width would seem logical.

Previous Canadian experimentation has shown that double-layered codends drastically reduced escapement of both haddock and cod. They have also shown that top chafing gear 10% wider than the codend affected escapement. A chafing gear somewhere between 10% wider

and 1¼ times as wide as the codend seems likely to be adequate.

To define precisely the minimum effective width may be impractical, since it may depend upon such variables as size of catch, length of codend bag, mesh size, and species of fish. Less extensive experimentation, however, might allow significant reduction in width of specified chafing gear.

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III. Cod Investigations in Subarea 2 - Labrador, 1950 to 1958

By A. W. MAY [1]

1. Introduction.

The cod sampling programme for Labrador during this period has been somewhat irregular, being carried on for the most part incidentally to other researches. A total of 1,448 pairs of otoliths were taken and 20,596 length measurements made. All samples were taken between mid-July and the end of September. One sample was jigged inshore in 15-20 fathoms and all others were taken offshore in depths ranging from 96 to 200 fathoms. The age readings and length measurements have been combined as shown below and in Figure 1.

Year	Subdivision	Gear	Otoliths	Length Meas.
1950	2G Inshore	Jigger	239	239
	2G Offshore	Trawl	110	251
	2H	Trawl	117	195
	2J	Trawl	118	579
1951	2J	Trawl	231	465
1952	2J	Trawl	None	2,233
1953	2J	Trawl	124	8,023
	2J	Longline	230	6,630
1954	2J	Trawl	120	450
1957	2J	Trawl	None	85
1958	2J	Trawl	159	1,446

2. Age Composition.

In the 1950 samples the 1942, 1944 and 1945 year-classes predominate, comprising together approximately 70% of each sample. The samples from 2G show a greater proportion of 6-year-old (1944 year-class) than 5-year-old fish (1945 year-class), while the reverse is true for the samples from 2H and 2J. There is a comparatively high number of 8-year-old fish (1942 year-class) in the offshore sample from 2G, and a high number of 5-year-old fish (1945 year-class) in the sample from 2J.

In 1951 the 1946 and 1947 year-classes appear in quantity (compared to 1950), and together make up 50% of the samples, with the 1947 year-class more abundant. The 1944 and 1945 year-classes are still present in fair quantity, but the 1942 year-class is not nearly as abundant as in 1950. The latter three year-classes now make up only 35% of the samples. As in 2H and 2J in 1950, the 1945 year-class is better represented than the 1944 year-class.

In the trawl sample of 1953, the age distribution is much the same as in 1951. The 1942, 1944, 1945, 1946 and 1947 year-classes together account for 88% of the sample. Again the 1947 and 1945 year-classes are better represented than those of 1946 and 1944, while the 1942 year-class is more evident than it was in 1951.

In contrast, in the longline sample the 1946 and 1947 year-classes are comparatively poor (14%), while the 1942 year-class is very prom-

[1] Fisheries Research Board of Canada, Biological Station, St. John's, Nfld.

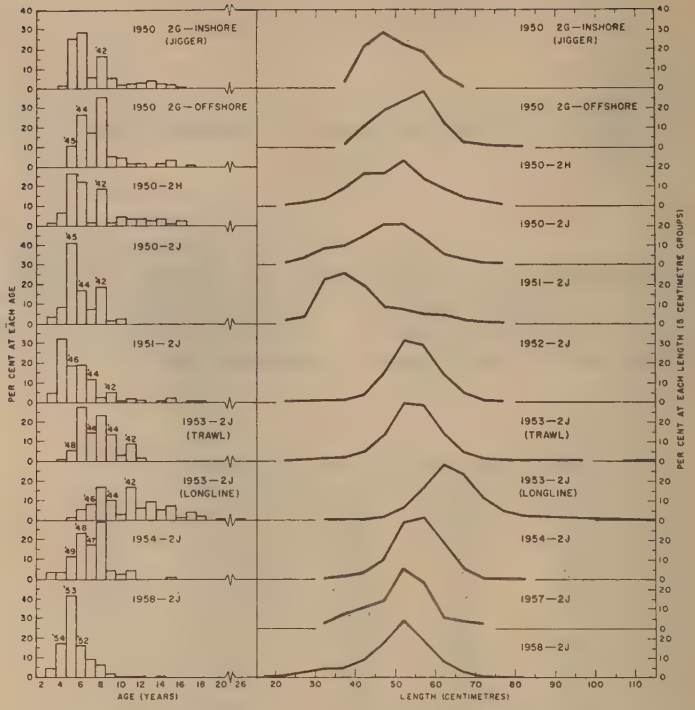

Fig. 1. **Age and length distributions of Subarea 2 cod combined by subdivision and method of capture. Length distributions are of all fish measured.**

inent (17%). The 1945 year-class is again more abundant than the 1944 year-class. The 1942, 1944 and 1945 year-classes are present in approximately the same total proportion in both the trawl and longline samples (46% and 44%). However, whereas most of the remainder of the trawl sample is composed of the 1946 and 1947 year-classes (42%), most of the remainder of the longline sample consists of fish of year-classes older than 1942 (37%).

In the **1954** sample the 1942 year-class is absent and the 1944 and 1945 year-classes are poorly represented. However, the 1946 and 1947 year-classes are still well represented (47%) and the 1948 and 1949 year-classes have appeared in fair numbers (35%). The 1946 and 1948 year-classes are predominant.

In the **1958** sample the 1953 year-class predominates (41.5%), followed by the 1954 (17.6%) and 1952 (16.4%) year-classes. The 1951 and 1950 year-classes combined account for 16% of the sample; all other year-classes are poorly represented or non-existent.

Summary and Conclusions.

Although the otolith samples were small, the trend from year to year allows some conclusions.

There are no strongly predominating year-classes, yet a fair amount of variability in year-class survival is evident. From 1942 to 1948 it appears that best survival occurred in 1942 and 1947, followed by comparatively good survival in 1944, 1945, 1946 and 1948. Survival in 1943 seems to have been very poor. The 1954 and 1958 samples are too small to permit valid conclusions to be drawn concerning year-class survival after 1948.

3. Length Distributions (Figure 1).

The length distribution of fish taken from 2G (inshore) in 1950 shows a peak in the 45-49 cm group, with a lesser peak in the 55-59 cm group, corresponding to the rich 1944 and 1945, and the 1942 year-classes respectively. In the offshore sample from 2G the peak is in the 55-59 cm group corresponding to the 1942 year-class. The length distributions from 2H and 2J also indicate the abundance of the 1945, 1944 and 1942 year-classes, though overlapping occurs. Here these three year-classes are represented by the large proportion of fish between 40 and 59 cm.

Most of the fish taken in 1951 are in the 30-49 cm range (1947, 1946 and 1945 year-classes), with the 1942 year-class evident in the 55-64 cm range.

In 1952 fish in the 50-59 cm range predominate. No age determinations were made but the length distribution indicates the presence in quantity of 6-to 8-year-old fish (1946, 1945 and 1944 year-classes).

It is evident from the 1953 age and length distributions that older and larger fish are taken by longline than by trawl. There is a predominance of fish of length 60-69 cm (mainly of the 1942 and older year-classes) in the longline samples, in contrast to a predominance in the 50-59 cm range (1947, 1946 and 1945 year-classes) in the trawl samples. Also, 23.5% of the fish taken by longline are above 70 cm in contrast to only 1.9% above 70 cm for trawl-caught fish.

In 1954 fish of length 50-59 cm predominate, corresponding to age-groups 6, 7 and 8 (1948, 1947 and 1946 year-classes).

In 1957 the peak is in the 50-54 cm group. This corresponds to fish of ages 6 and 7 (1951 and 1950 year-classes). However, the length distribution is probably not reliable, being of 85 fish only.

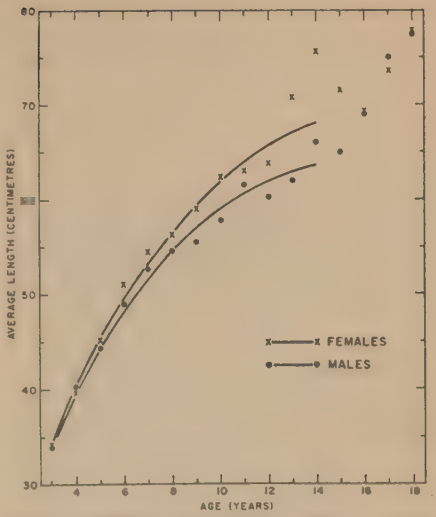

Fig. 2. Subarea 2 cod, 1950 to 1958. Growth curves for males and females, ages 3 to 18 years.

In 1958 the peak is in the 50-54 cm group, again pointing to a predominance of 6-and 7-year-old fish (1952 and 1951 year-classes). This probably gives a better picture of the age composition than was obtained from otolith readings, since the number of fish from which otoliths were taken (159) is small in comparison to the number represented in the length distribution (1,446).

4. Growth.

Although some variation in growth was evident from year to year, and from one area to another, the samples were considered too small to allow any conclusions as to the nature and causes of these variations. The average growth curves for males and females, for all years and subdivisions combined, are shown in Figure 2. The growth of females exceeds that of males from the fifth year.

5. Sex Composition.

The females predominate in all but one of the samples. The percentage of females varies between 51.6% and 68.3% of the samples except in 1958 (49.1%).

CANADIAN PACIFIC OCEANOGRAPHY SINCE 1953

JOHN P. TULLY

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In Canada, oceanographic research is supported by the Federal Government through the Canadian Joint Committee on Oceanography. This Committee includes representation from the Fisheries Research Board of Canada which provides scientific staff, research equipment, laboratories, and administration at Nanaimo, British Columbia; from the Royal Canadian Navy which provides and operates two full-time ocean-going research vessels on the Pacific coast; the Hydrographic Service which undertakes auxiliary and tidal observations along with hydrographic surveys; the Meteorological Service which provides facilities in the weatherships on ocean weather station PAPA (latitude 50° N., longitude 145° W.); the Defence Research Board of Canada which provides logistic and material support; and the National Research Council of Canada which provides material assistance. The programs are directed toward the immediate and long-term needs of these supporting members of the Joint Committee. Close liaison is maintained with all agencies, domestic and foreign, having an interest in the oceanography of the north Pacific Ocean.

During the years since the last Congress (1953), the Canadian observational and research effort in the Pacific has increased considerably. Studies of the larger coastal seaways and of the northeast Pacific Ocean have been undertaken; and, in addition, fundamental studies in physics and chemistry have been initiated. The use of hydraulic models as an oceanographic tool has continued, and at the present time the tidal features of a large coastal region are being studied through this medium. Computers of various types are being used as aids in processing and interpreting field data and in theoretical problems. A considerable proportion of the effort has been devoted to observations of the properties of the water from surface to 1,500 meters depth, and interpretation of these data to describe the structure, mechanism, and biotic productivity of the waters of interest to Canada.

In the summer of 1955, oceanographic agencies from the United States, Japan, and Canada cooperated in Project Norpac. This was a synoptic survey of the Pacific Ocean north of latitude 20° N. Twenty ships took part and about 1,000 stations were occupied during the month of August. The data were processed, published, and the data records exchanged in Honolulu in February, 1956, five months after completion of the sea work. This was the most comprehensive survey ever undertaken in any oceanic area.

Since Norpac, the stimulus for oceanic study has been provided by the International North Pacific Fisheries Commission. Under this protocol, Canada has carried out extensive exploratory fishing and oceanographic observations through the summers of 1956 and 1957. In support of this work, comprehensive oceanographic surveys have been made in the oceanic area north of latitude 40° N. and east of longitude 170° W., in August, 1956, and in February and August, 1957.

Time series observations are taken from the weathership at ocean weather station PAPA (latitude 50°N., longitude 145° W.) to monitor the changing conditions in mid-ocean. These include semi-daily bathythermograph observations, which are continuous since 1952. In 1956, an oceanographer was placed on one of the two ships which occupy the station for alternate six-week periods. This provides bi-weekly observations of the properties of the water from surface to 1,500 meters depth, and plankton to 300 meters depth.

In conjunction with these oceanic programs, drift bottles, in lots of 1,000, have been released every six weeks from the weathership, and at four positions across the area of each survey. A one dollar reward is paid to the finder for return of the enclosed information card with date and place of recovery. The cards are printed in English, Spanish, Japanish, and Russian.

Detailed surveys are now being carried out in the waters over the continental shelf and slope and for a distance of 200 miles to seaward, five to six times a year. This program provides the description of the continental periphery of the oceanic system and describes the region of active Canadian fisheries. In support of this program, daily observations of surface seawater temperature and salinity are made at four lightstations

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along the ocean coast. These provide continuous data necessary to link the intermittent data from the surveys. They are continuous since 1935 and provide a means of evaluating oceanographic conditions in the past as well as in the present.

Oceanographic exploration of the inshore seaways has been completed. The last such project was in the sea area between the Queen Charlotte Islands and the mainland, where surveys were carried out at bi-monthly intervals for nearly two years. Daily seawater observations from lightstations are made at strategic points in all coastal seaways to provide a continuing monitor of the conditions which have been defined by the surveys.

Recently the techniques of seawater analyses and the tedious calculations in the data have been mechanized. Salinity is determined at sea with precision conductivity equipment to a higher degree of accuracy than was previously possible in a shore laboratory with standard chemical apparatus. All computations are carried out by a digital computer. The data can now be published in a manuscript (multilith) series, within one month of the conclusion of sea operations.

Research is being undertaken in the field of biotic productivity. The amount of organic carbon in the plankton is determined as the measure of the standing crop. Carbon fourteen studies are being undertaken to determine the rate of productivity. The nutrient content of the water is being determined by modernized methods. The role and importance of the various nutrients and light in the sea is being undertaken. All these lead to assessment of the fertility in the sea and its controlling factors.

The physical program is being extended to consider the heat exchange between sea and atmosphere to determine the manner and rate of the heating and cooling processes. Studies of abyssal water are being undertaken to determine their source, rate of movement, and mechanics of their entrainment into the surface waters. This research is also of importance to the prediction of pollution from nuclear wastes disposed in the deep ocean.

Hydraulic models have been used successfully to study the tidal and flushing mechanisms of coastal seaways where the boundary conditions are known. These models also assist materially in the appreciation and interpretation of sea data and indicate regions of importance in the area of study. The most recent project is a model of the sea area between the Queen Charlotte Islands and the mainland, to reveal the tidal currents and circulation system, which could not be economically resolved from sea data. The model is 30 meters long by 10 meters wide representing an area of 70,000 square kilometers. It has a highly distorted length to depth ratio. Fresh and sea water are used to reproduce the density gradients. Tides are predicted by an electronic computer and imposed on the model mechanically. The tidal rise at points in the area is recorded and compared to the predicted tides. The tidal currents are observed by photographing the movement of dye patches in the model.

Oceanographic education at the postgraduate level is offered at the Institute of Oceanography of the University of British Columbia at Vancouver, British Columbia. Candidates having a B.A. degree may proceed to a Master's degree in chemistry in the sea, or to a Master's or Doctor's degree in physics or biology in the sea. The facilities provide for two years of lecture courses and opportunities for research and sea experience. Registration has been encouraging, and graduates have been readily placed in responsible positions in oceanographic research.

Data from oceanographic surveys and experimental work are published in a manuscript report series which is circulated to the principal laboratories interested in north Pacific studies. Interim results of continuing research are also published in this series, but conclusive results are published in the *Journal of the Fisheries Research Board of Canada* or in journals suitable to the topic.

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CANADIAN OCEANOGRAPHIC RESEARCH IN THE NORTHEAST PACIFIC OCEAN

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The region of the Pacific Ocean of primary interest to Canada includes the area north of latitude 40°N. and east of longitude 175°W. This is the ocean habitat of the American and Canadian salmon stocks. Synoptic oceanographic surveys have been carried out in this area during three summers, August, 1955, 1956 and 1957, and one winter, February, 1957. The first survey was "Project Norpac" in which fifteen oceanographic agencies in Japan, the United States, and Canada made coordinated surveys over the whole ocean north of latitude 20°N. The subsequent surveys have been undertaken by the Canadian Government as part of its obligation to the research program of the International North Pacific Fisheries Commission. Each survey required about six weeks sea time and was made during the period of maximum summer, or minimum winter temperatures, when oceanographic conditions were most nearly stable. The properties of the water, the structure, and the currents from the surface to more than 1,000 meters depth and frequently to 2,000 meters were examined.

All these data have been published in Data Records, in the Manuscript Series of the Fisheries Research Board of Canada.

In addition to these surveys, extensive exploratory fishing for Pacific salmon has been carried out, during which oceanographic observations were made from the fishing vessels.

From these data it is now possible to present the principal oceanographic features of this area, the current systems, and the summer and winter variations. No attempt is made to discuss the oceanographic variations that occur from summer to summer. Furthermore, it would be very laborious to review all the data here. This is not necessary since it has been and is being displayed elsewhere *(7)*. It is sufficient to illustrate these discussions with examples from one summer and one winter survey, and comment on the similarities and differences observed in other surveys.

TEMPERATURE

The observed distribution of temperature for August, 1955, over the surface and in south to north vertical sections is shown in Fig. 1. These data show that there is a gradient of temperature from latitude 40°N. in mid-ocean, northward towards the North American Coast. A minimum temperature occurs in the western part of the Gulf of Alaska. From this cold center, the temperatures increase shoreward. South of latitude 40°N., in mid-ocean, there is a central body of warm water.

The longitudinal sections show that the temperatures decrease with depth. There is an upper, warm layer of the order of 50 meters depth. Below this is a well defined thermocline extending to about 100 meters, and then generally a gradual gradient of decreasing temperature. Below the thermocline there are small irregularities in the temperature structure that occur between depths of 120 to 200 meters, the magnitude of the temperature inversions and the depths at which they occur vary within the area. These anomalies occur in the region of the halocline. The temperatures decrease to the northward in the upper layers and in the depths so that the temperature gradient in the thermocline remains nearly constant through the area. In the deep water the isotherms rise toward the surface, going from south to north, and come nearest to the surface in the region of coldest surface water in the Gulf of Alaska. However, the thermocline remains continuous and at constant depth through this cold center, separating the cold surface water from the dome of cold deep water.

In later years, 1956 and 1957, these principal features persisted. However, as shown in Fig. 2 the cold center moved westward, and the isotherms curved northeastward around the Gulf of Alaska rather than southwestward.

Figure 3 shows the winter data observed in February, 1957. Over the surface (10 meters depth), the configuration of the isotherms was generally similar to those observed in summer. The highest temperatures were at the southern limit of the region surveyed, and the coldest water was in the Gulf of Alaska, south of Kodiak and the Shumigan Islands. It is notable that the winter

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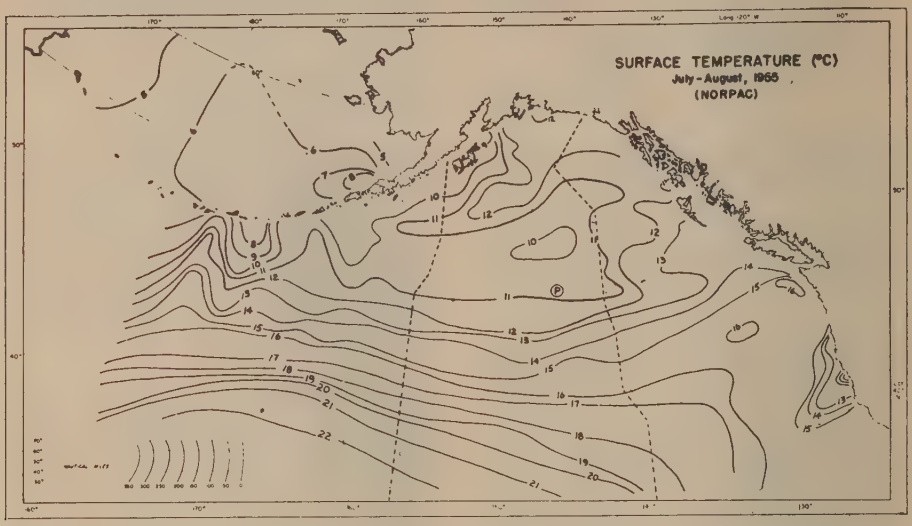
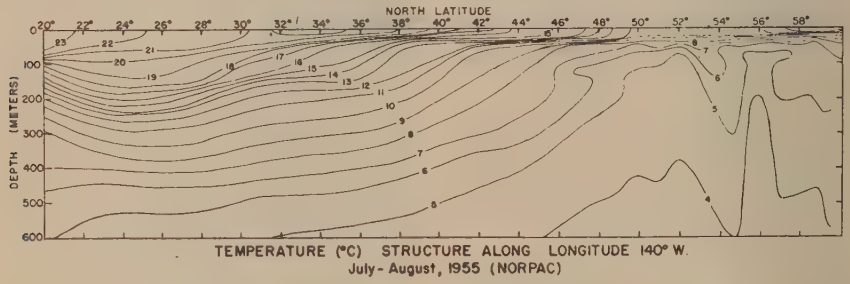
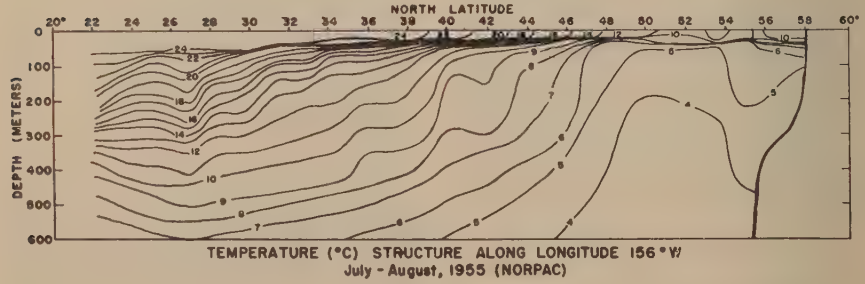




TEMPERATURE (°C) STRUCTURE ALONG LONGITUDE 140° W.
July - August, 1955 (NORPAC)



TEMPERATURE (°C) STRUCTURE ALONG LONGITUDE 156° W
July - August, 1955 (NORPAC)

Fig. 1.—

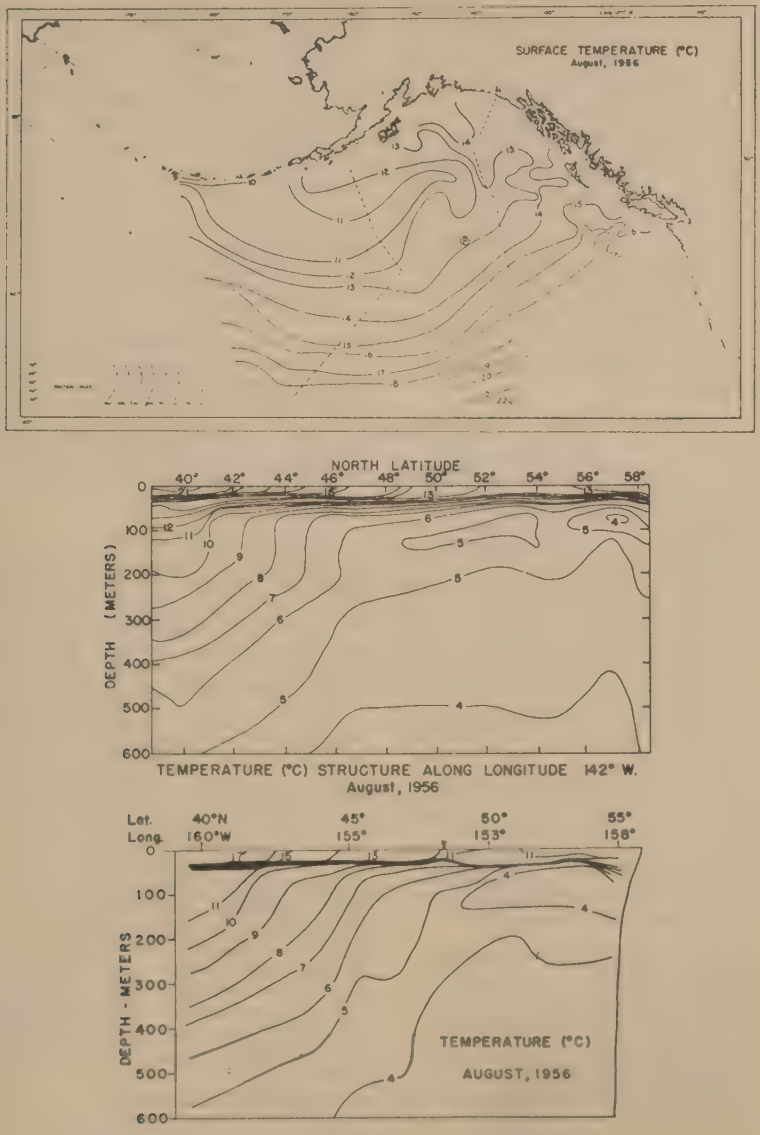

SURFACE TEMPERATURE (°C)
August, 1956
NORTH LATITUDE
40° 42° 44° 46° 48° 50° 52° 54° 56° 58°
DEPTH (METERS)
0 100 200 300 400 500 600
TEMPERATURE (°C) STRUCTURE ALONG LONGITUDE 142° W.
August, 1956
Lat. 40°N 45° 50° 55°
Long. 160°W 155° 153° 158°
DEPTH - METERS
TEMPERATURE (°C)
AUGUST, 1956

Fig. 2.—

range of temperature (4° - 11°C.) over the area was almost identical with that observed in summer, from 11° to 18°C. This suggests that the total heating and cooling was very nearly constant over the whole area and amounted to about 7°C.

The longitudinal sections show that there was a south to north gradient in the surface and deep waters, with the coldest region in the Gulf of Alaska. In the area extending about 250 miles offshore, the surface waters were cold. The temperature was isothermal or decreased slightly to a depth of 125 meters. The temperature then increased to a maximum at about 150 to 175 meters, and again decreased regularly with depth. This temperature inversion was stable because it coincided with the halocline. Over the remainder of the area the upper waters were warmer than the deep waters, and the thermocline became pronounced to southward.

Hollister *(1)* examined the daily bathythermograph observations from ocean weather station PAPA (latitude 50° N., longitude 145° W.) through the years 1950 to 1955. From this study a representative diagram of the annual temperature cycle and representative gradients are shown in the upper diagram of Fig. 4. In mid-winter (February - March), the upper waters are isothermal and somewhat colder than the deep waters. Spring warming becomes apparent in April when a thermocline develops at about 50 meters depth. This thermocline becomes more intense and deepens as the warming continues through September. Surface cooling starts in late September and continues through the autumn while the thermocline becomes deeper and less marked until it vanishes in January. The annual temperature range at the surface is seven or more degrees Centigrade. This decreases with depth to 120 meters where the range is less than one degree. These observations were confirmed by Robinson *(3)*. She showed that the temperature cycles and range in all parts of the area were similar to that shown in Hollister's figure. Of course, the absolute values varied according to position.

These data show that the annual temperature cycle is consistently recurrent, the range of seasonal variation is nearly constant over the area, and that the temperatures below the deepest level of the thermocline are nearly constant. It is inferred from this that the depth of the thermocline marks the limit of seasonal heating and cooling, and that the temperature distribution in the deep water is independent of the cycle of the upper zone.

There is no doubt that one of the principal features of these sub-Arctic regions is the annual cycles of heating and cooling. The amount of heat directed toward the sea depends principally on the mean altitude of the sun and the hours of daylight. These vary with latitude and season. The amount of heat reaching the sea is reduced by absorption in the atmosphere, reflection from the sea surface, and cloud cover. The sea loses heat by back radiation, transfer of sensible heat to the atmosphere, and evaporation. These processes were studied in the British Columbia coastal waters by Tabata *(5)* who derived the net heat transfer as shown in the second diagram of Fig. 4.

From the end of March until the end of September, the heating processes are dominant. During the remainder of the year, the waters are being cooled. This corresponds precisely with the observed temperature cycle shown in the first diagram where maximum temperatures occur in September at the end of the heating period, and minimum temperatures occur in late March at the end of the cooling period. This suggests that the heating and cooling of the surface water is primarily a local process.

Figure 5 shows the annual cycle of daily incident energy from the sun through the northern temperate latitudes. During the summer, the incident energy is nearly constant from latitude 40° N. to 60° N., a gradient develops in autumn, reaches a maximum in winter, and degenerates in spring. The summer and winter gradients of incident energy accompany the diagram.

The second pair of diagrams show the average cloud cover over the area in summer and winter *(8)*. Cloud cover absorbs radiant energy at the rate of 7.1% for each tenth of the sky obscured. Applying this correction to the radiant energy, the average of seasonal insolation over the area was obtained as shown in the third pair of diagrams. This corresponds remarkably with the summer and winter surface isotherms shown in the last two diagrams of the figure. This is further confirmation that heating is primarily determined by insolation, and the cooling factors are relatively constant through the year. It is also apparent that in the absence of a longitudinal heating gradient, as in summer, the cloud cover is a major feature; however, in the winter the cooling processes are dominant, and the cloud cover is a secondary feature.

This analysis has not been completed to determine if the heat content of the upper zone corresponds precisely to the heat budget, or why the

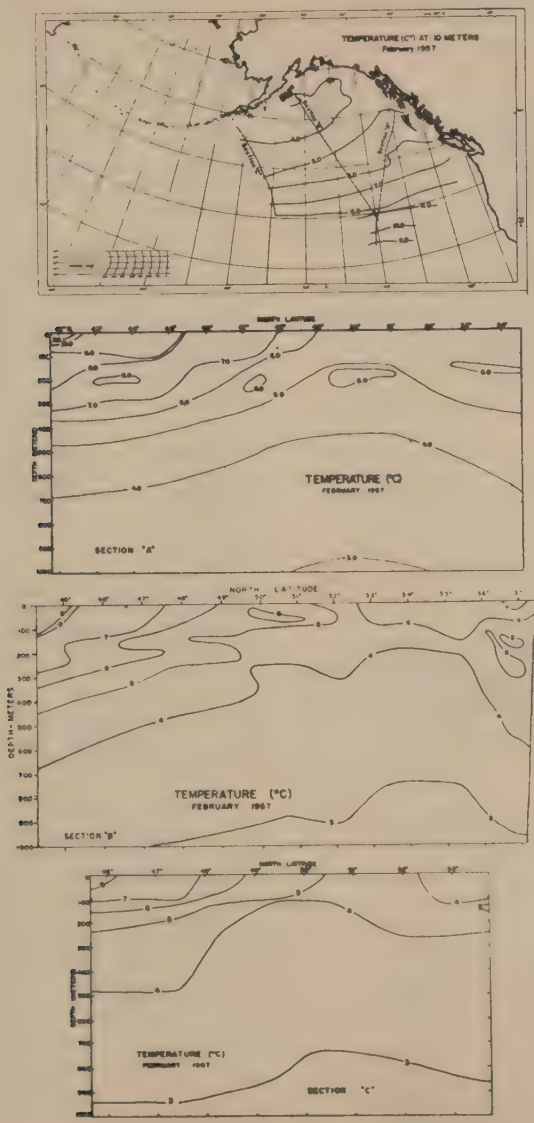

TEMPERATURE (C°) AT 10 METERS
February 1957
TEMPERATURE (°C)
FEBRUARY 1957
SECTION "A"
NORTH LATITUDE
DEPTH-METERS
TEMPERATURE (°C)
FEBRUARY 1957
SECTION "B"
TEMPERATURE (°C)
SECTION "C"

Fig. 3.—

annual temperature range is nearly constant over the area. Such an examination is necessary to fully understand the phenomena.

SALINITY

The distribution of salinity over the surface, and in longitudinal section in the August survey of 1955 is shown in Fig. 6. There was a general south to north gradient over the surface. The highest salinities occurred in mid-ocean in the sub-Tropic latitudes and decreased north and east toward the North American Continent. This corresponds to the mid-ocean temperature gradient, and like it, may be regarded as a boundary between the sub-Tropic and sub-Arctic waters.

A second feature of the diagram is the plateau or secondary maximum of salinity ($32.8°/_{oo}$) in the Gulf of Alaska. Around this the salinity decreased shoreward. This salinity maximum corresponded approximately to the temperature minimum.

The longitudinal sections are most striking. Southward of latitude 36°N., the surface salinities exceeded $34°/_{oo}$ and became less with depth to a minimum between 400 and 500 meters. This is typical of the Tropic and sub-Tropic regions. North of latitude 36°N., the surface salinities were less than $33.8°/_{oo}$ at the surface and increased with depth.

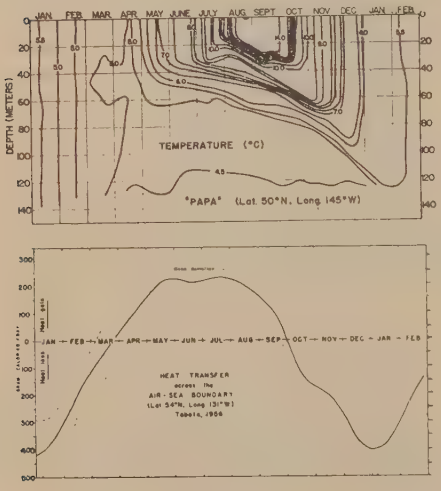

JAN FEB MAR APR MAY JUNE JULY AUG SEPT OCT NOV DEC JAN FEB
DEPTH (METERS)
TEMPERATURE (°C)
"PAPA" (Lat. 50°N. Long. 145°W)
JAN — FEB — MAR — APR — MAY — JUN — JUL — AUG — SEP — OCT — NOV — DEC — JAN — FEB
HEAT TRANSFER
across the
AIR - SEA BOUNDARY
(Lat 54°N, Long 131°W)
Tabata, 1956

Fig. 4.—

In this northern sub-Arctic region there is an upper zone of relatively homogeneous low salinity water, less than $33.0°/_{oo}$ extending to about 100 meters depth. Below this is a halocline extending to 175 to 200 meters depth in which the salinity increases to $33.8°/_{oo}$. This halocline is a boundary separating the upper low-salinity zone from the deep regions where the salinity is greater than $33.8°/_{oo}$ and increases gradually with depth. These sequences of structures along the section are illustrated in the figure.

The salinity gradients in these sections do not correspond to the temperature gradients. The south-north surface gradient marks the separation of the sub-Tropic and sub-Arctic surface waters. The vertical gradients separate the upper and lower zones. However, between latitudes 34° and 38° these are not distinguished. These are the latitudes of the so-called Polar Front as defined by the surface temperature isotherms.

Figure 7 shows similar data observed a year later, during August, 1956. The general situation was the same as in the previous year. The boundary between sub-Arctic and sub-Tropic waters was somewhat farther south, and the surface maximum in the Gulf of Alaska had a greater extent.

The winter data are shown in Fig. 8. Within the limits of normal variation, the distribution of salinity in this area was the same as in summer. That is, the salinity structure, unlike the temperature structure, is constant throughout the year.

In these figures it is notable that the halocline like the thermocline is continuous and at constant depth through the center of maximum surface salinity.

Jacobs *(2)* (Fig. 9) has shown that the low surface salinity in this area is due to the excess of precipitation over evaporation. Northward of the Polar Front there is an accumulation of fresh water in the upper zone of the ocean. The extent of this accumulation is indicated in Fig. 10. The first diagram shows a "logarithmic plot," after Tully *(6)*, of the salinity at a typical station. Examination of the Canadian part of the Norpac (August, 1955) data showed that the bottom of the halocline was defined by the isohaline $33.77°/_{oo} \pm 0.04°/_{oo}$. This was defined as the depth of the upper zone as shown in the third diagram of the figure. These data were then transferred to a natural plot (second diagram) where the dilution of the upper zone was attributed to some depth of fresh water contained in a column of unit cross-section, which had been mixed with the

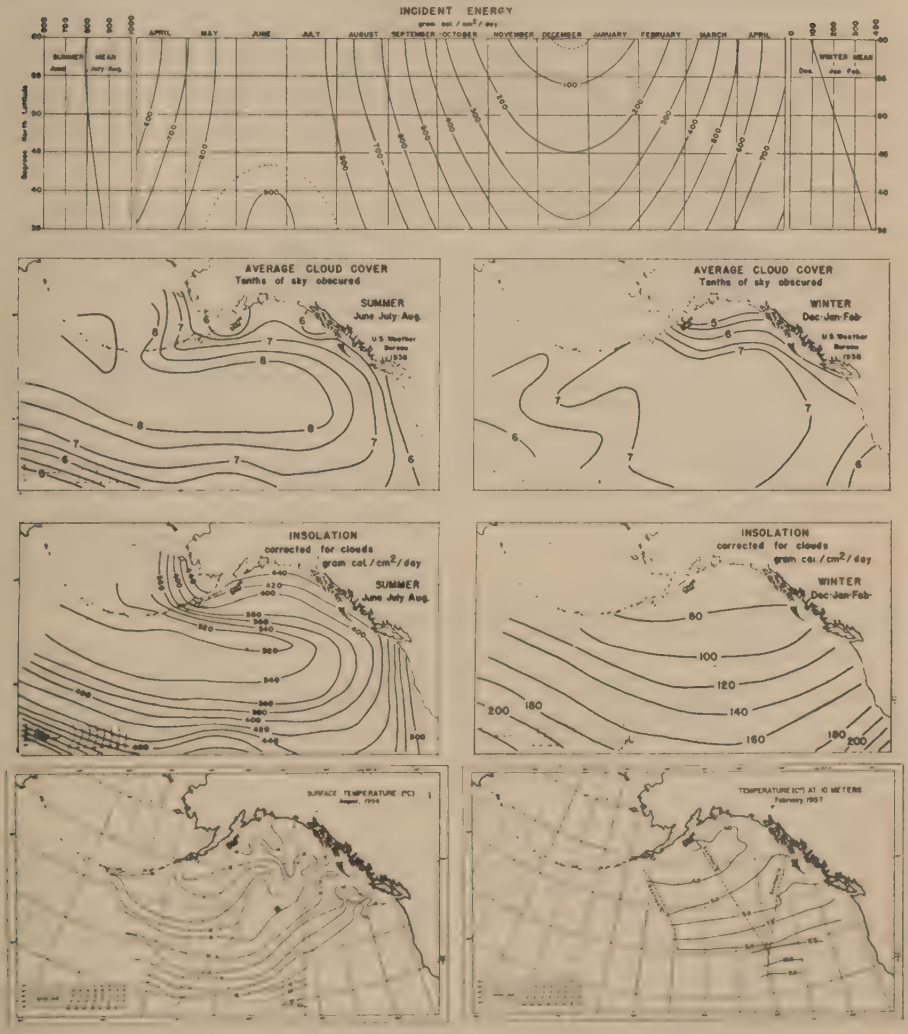

INCIDENT ENERGY
gram cal / cm² / day
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY
FEBRUARY
MARCH
APRIL
SUMMER
June
NEAR
July Aug.
WINTER NEAR
Dec.
Jan Feb.
Degrees North Latitude
AVERAGE CLOUD COVER
Tenths of sky obscured
SUMMER
June July Aug.
U.S. Weather Bureau
1938
AVERAGE CLOUD COVER
Tenths of sky obscured
WINTER
Dec Jan Feb
U.S. Weather Bureau
1938
INSOLATION
corrected for clouds
gram cal / cm² / day
SUMMER
June July Aug.
INSOLATION
corrected for clouds
gram cal / cm² / day
WINTER
Dec Jan Feb
SURFACE TEMPERATURE (°C)
August, 1934
TEMPERATURE (C°) AT 10 METERS
February 1937

Fig. 5.—

underlying sea water of salinity 33.77‰ to the depth of the upper zone.

This depth of fresh water in the upper zone of the principal part of the sub-Arctic region varies from 2.5 meters in the vicinity of salinity maximum in the Gulf of Alaska to a maximum of 5.5 meters near the coast. There is reasonable agreement between this and the previous assessment of excess precipitation considering that this is a specific picture (August, 1955) and the former (Fig. 9) is an average based on seasonal data over a long period of years.

The average accumulation of fresh water is about 3.5 meters while the average annual contribution is about 0.6 meters (60 centimeters). Hence, it may be reasoned that there is about six years accumulation of fresh water in this part of the North Pacific Ocean.

A cursory examination of the Japanese data immediately to the westward between latitudes 40° N. and 55° N. indicates that the depth of fresh water is about 2.3 meters. This difference of 1.2 meters represents about two years accumulation of fresh water in the eastern North Pacific over that present in the western area.

WATER MASSES

One convention for describing water masses is the temperature-salinity (T-S) diagram. In this the waters are identified by the relation of the two properties without reference to depth. Fig. 11 shows a sequence of representative curves, numbered for convenient reference, and the regions associated with their occurrence. The sequence of numbers was chosen to allow for extension of the study into adjacent regions. These curves represent a family of gradually changing properties throughout the northeast Pacific and are arbitrarily chosen to represent successive stages in the character of the water. Except where two or more contours come together, there are no marked boundaries, rather the properties vary from one type to the next through considerable distance.

The deep zone is similar within small limits over the whole area. However, more careful examination may show that the differences in the deep waters are significant.

The principal differences are in the upper zone. The first three curves (10, 11, and 12) denote the sub-Arctic and associated waters in which the thermocline and halocline are distinct. It has been shown that the thermocline vanishes in winter, when the upper zone structure is dependent on salinity alone. The extreme sub-Arctic water is evidently associated with the Bering Sea and the Oyashio. It is marked by minimum temperatures and a distinct temperature inversion in the halocline. In the eastern ocean the temperature of the halocline rises, the inversion is reduced or vanishes, but the salinity characteristics remain generally similar.

The other extreme is the sub-Tropic water (Type 15) in which the thermal and salinity gradients are coincident, and persist throughout the year. It is further marked by a salinity minimum which has already been discussed. This water is generally associated with the Kuroshio extension and mid-ocean circulation.

Types 13 and 14 are intermediate between the two principal water masses. Type 13 shows two thermoclines, separated by an intermediate halocline. Only the upper thermocline vanishes in winter. Type 14 represents the so-called isohaline region where the salinity is markedly irregular, and may show as many as four inversions in the upper zone.

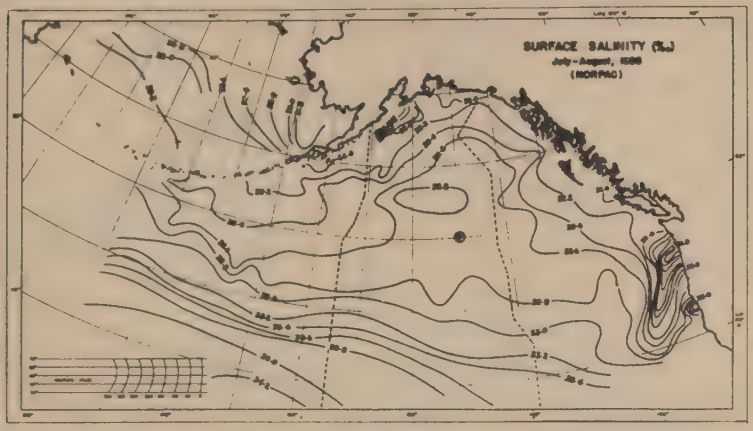
The principal boundary in the region is between the sub-Arctic and sub-Tropic waters. This boundary is most marked in mid-ocean and was more pronounced in 1955 than in 1956. Near the North American Coast the boundary region becomes broad and indistinct.

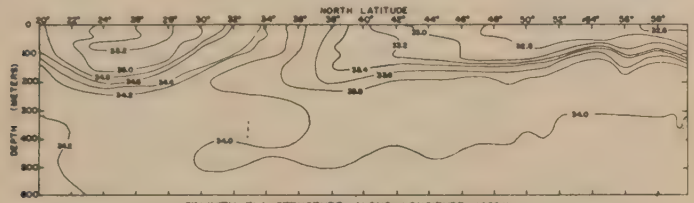
The only secondary boundary noted to date is between Types 9 and 10. The Type 9 water lying westward of longitude 180° is markedly more saline than the Type 10 water, and the transition does not fit the sequence of salinity. This is associated with a dynamic boundary.

CURRENTS

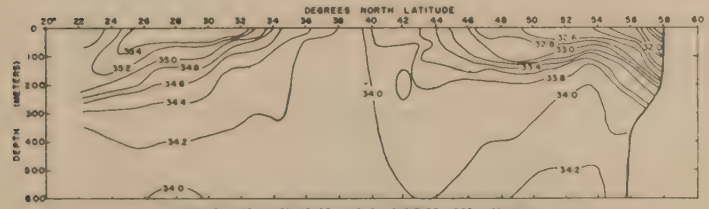
The geopotential topography in August, 1955, is shown in Fig. 12. The contours show the variations of sea surface referred to a presumed equipotential surface at 1,000 meters depth. In accord with the geostrophic principles, the current is presumed to flow along the contours with velocity proportional to the gradient across them.

The principal features of physical structure are reflected here. In the Polar Front there is a broad movement progressing eastward at less than two miles per day. The southern side of this current progresses to the American Coast where it turns southward to join the Tropical circulation system, and complete the mid-ocean circulation. The northern side of the current veers northward

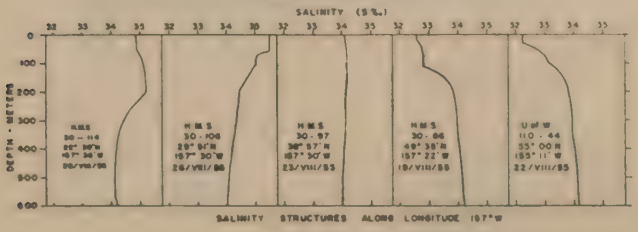




SALINITY (‰) STRUCTURE ALONG LONGITUDE 140° W.
August, 1955



SALINITY (S‰) STRUCTURE ALONG LONGITUDE 156° W (Appros)
July - August, 1955 (NORPAC)



SALINITY STRUCTURES ALONG LONGITUDE 157°W

Fig. 6.—

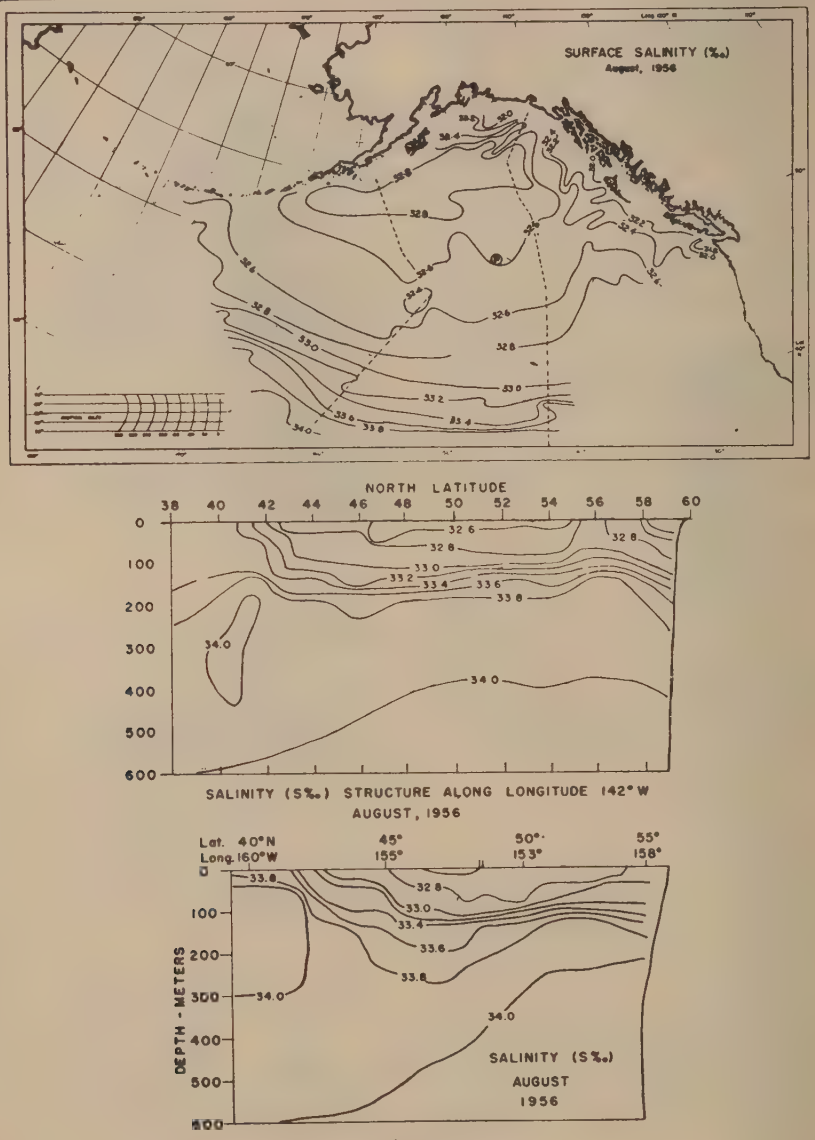

SURFACE SALINITY (‰)
August, 1956
NORTH LATITUDE
38 40 42 44 46 48 50 52 54 56 58 60
0 100 200 300 400 500 600
32.6
32.8
33.0
33.2
33.4
33.6
33.8
34.0
SALINITY (S‰) STRUCTURE ALONG LONGITUDE 142° W
AUGUST, 1956
Lat. 40°N Long. 160°W
45° 155°
50° 153°
55° 158°
DEPTH - METERS
SALINITY (S‰)
AUGUST
1956

Fig. 7.—

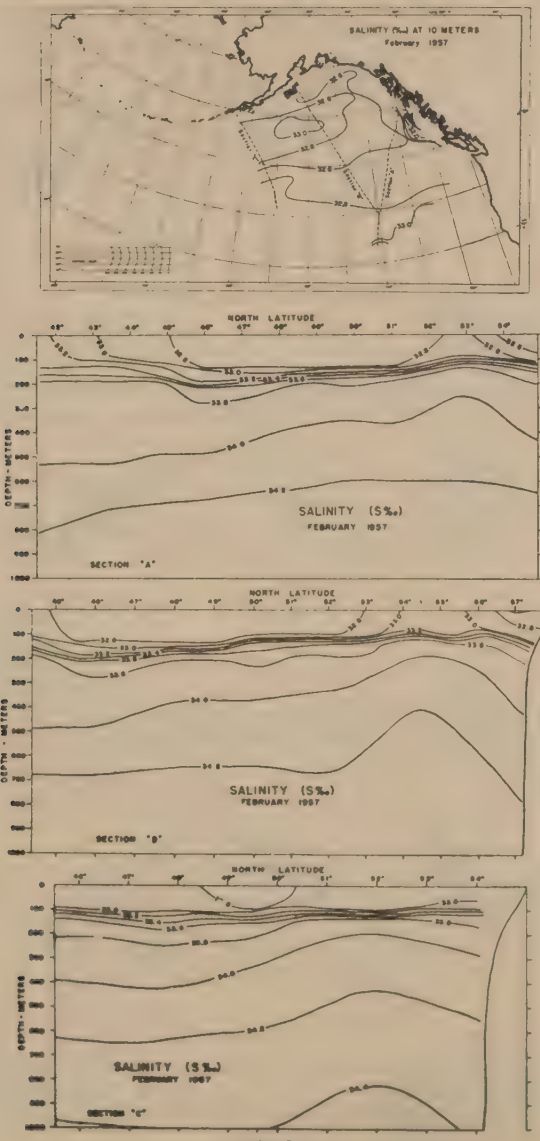

SALINITY (‰) AT 10 METERS
February 1957
NORTH LATITUDE
DEPTH - METERS
SALINITY (S‰)
FEBRUARY 1957
SECTION "A"
SECTION "B"
SECTION "C"

Fig. 8.—

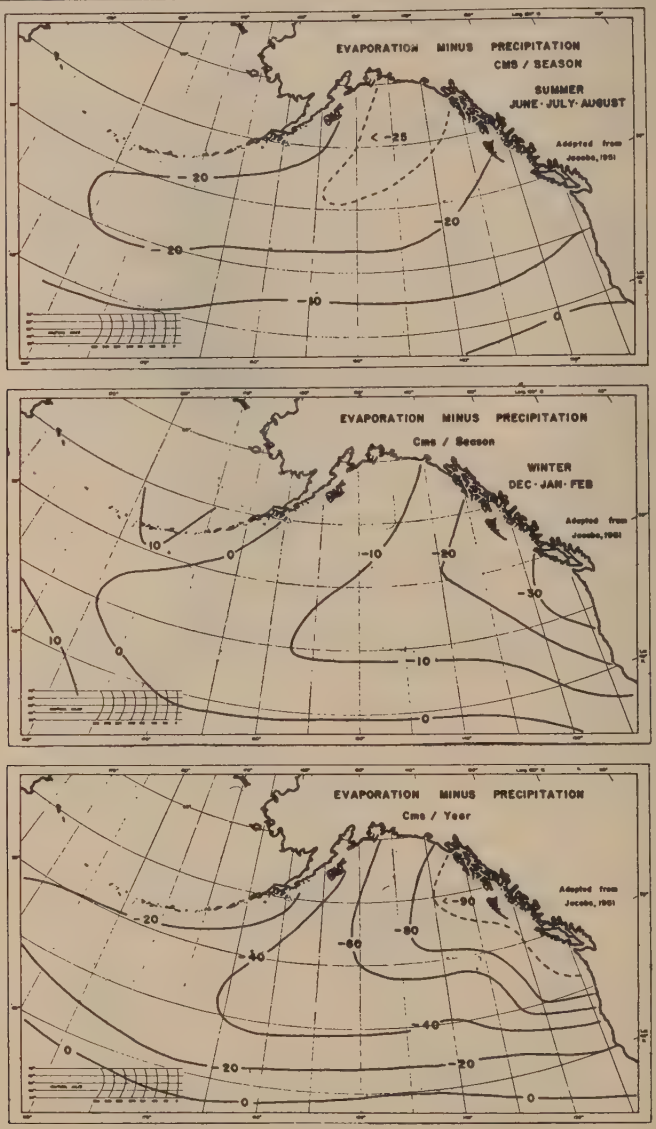

EVAPORATION MINUS PRECIPITATION
CMS / SEASON
SUMMER
JUNE · JULY · AUGUST
Adapted from Jacobs, 1951
< -25
-20
-20
-20
-10
0
EVAPORATION MINUS PRECIPITATION
Cms / Season
WINTER
DEC · JAN · FEB
Adapted from Jacobs, 1951
10
0
-10
-20
-30
10
0
-10
0
EVAPORATION MINUS PRECIPITATION
Cms / Year
Adapted from Jacobs, 1951
-20
-40
-60
-80
<-90
-40
0
-20
-20
0
0

Fig. 9.—

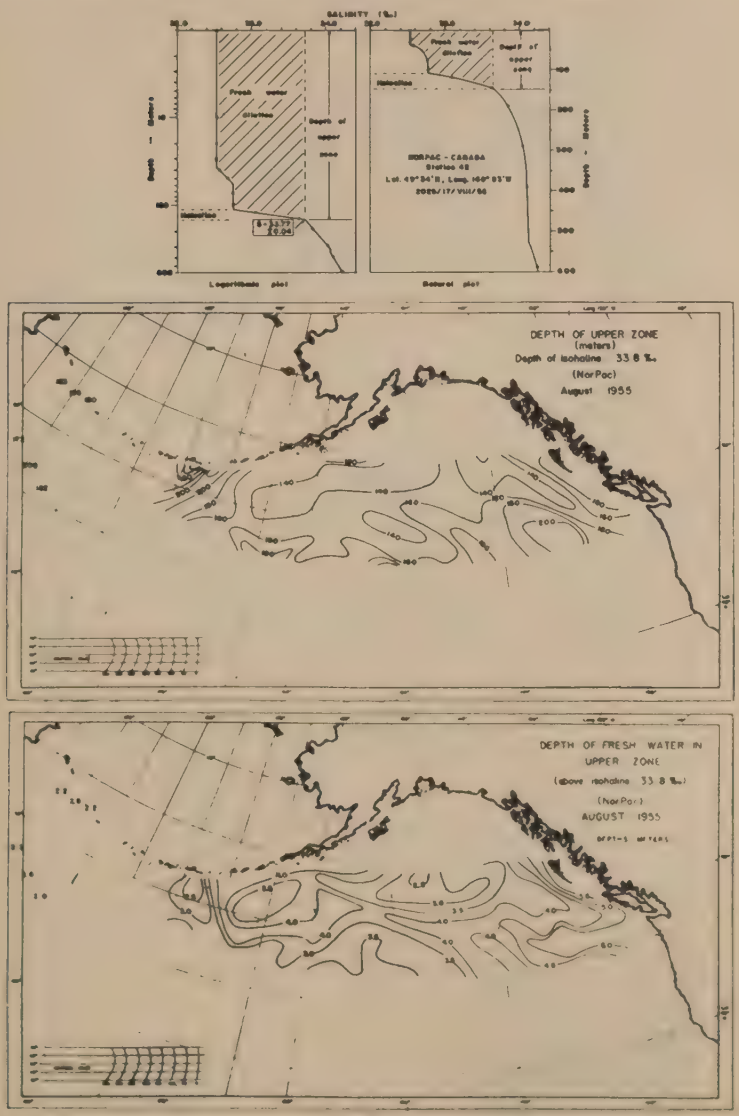

SALINITY (‰)
Fresh water
Depth of upper zone
Isohaline
Depth - Meters
Logarithmic plot
Natural plot
DEPTH OF UPPER ZONE
(meters)
Depth of isohaline 33.8 ‰
(NorPac)
August 1955
DEPTH OF FRESH WATER IN
UPPER ZONE
(above isohaline 33.8 ‰)
(NorPac)
AUGUST 1955

Fig. 10.—

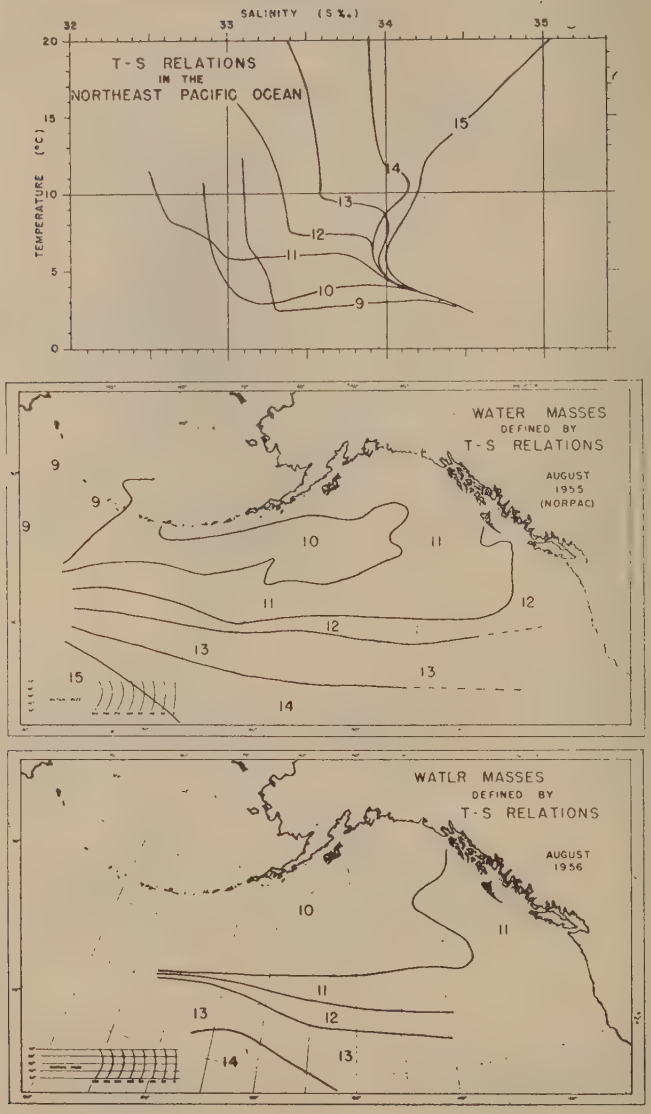

SALINITY (S‰)
32
33
34
35
20
15
10
5
0
TEMPERATURE (°C)
T-S RELATIONS
IN THE
NORTHEAST PACIFIC OCEAN
15
14
13
12
11
10
9
WATER MASSES
DEFINED BY
T-S RELATIONS
AUGUST
1955
(NORPAC)
9
9
9
10
11
11
12
12
13
13
15
14
WATLR MASSES
DEFINED BY
T-S RELATIONS
AUGUST
1956
10
11
11
12
13
14
13

Fig. 11.—

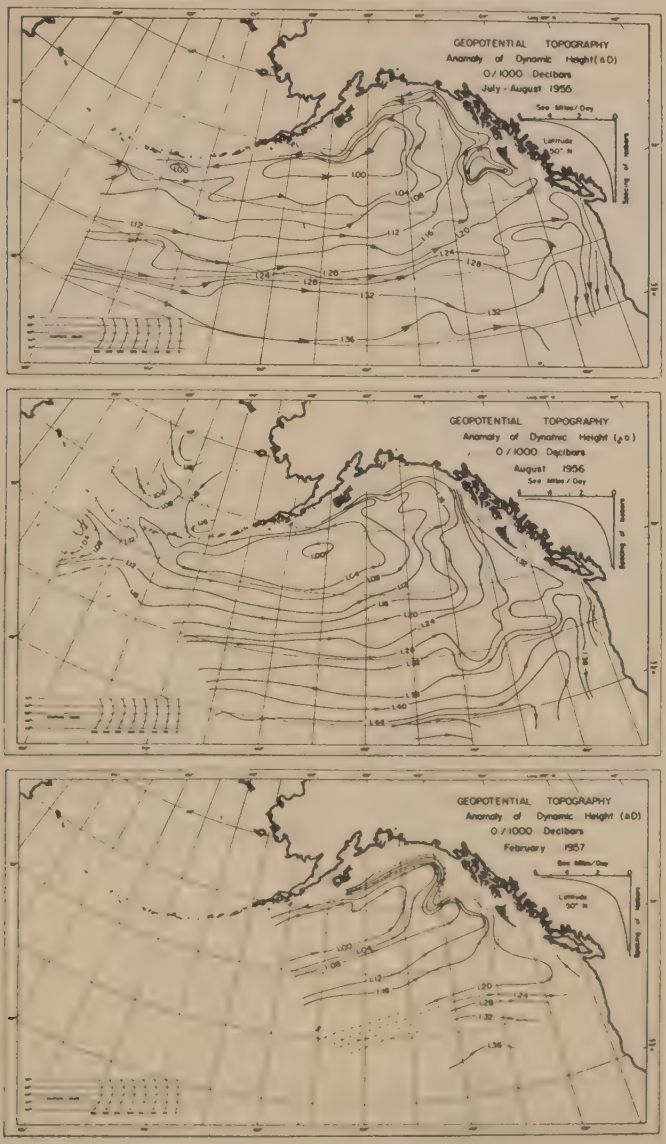

GEOPOTENTIAL TOPOGRAPHY
Anomaly of Dynamic Height (ΔD)
0 / 1000 Decibars
July - August 1955
GEOPOTENTIAL TOPOGRAPHY
Anomaly of Dynamic Height (ΔD)
0 / 1000 Decibars
August 1956
GEOPOTENTIAL TOPOGRAPHY
Anomaly of Dynamic Height (ΔD)
0 / 1000 Decibars
February 1957

Fig. 12.

around the Gulf of Alaska and is strongly intensified along the Alaska Peninsula. The contours indicate that very little of this water is recirculated; therefore, it is deduced that the current moves eastward close along the Aleutian Islands and is dissipated through the passages into the Bering Sea. There is some evidence in the figure of a strong northward set in the vicinity of longitude 180° and a cyclonic gyral in the western Bering Sea. Earlier studies in 1947*(4)* showed a northward and westward movement in the eastern Bering which accords well with these observations.

The diagram suggests that the sub-Arctic waters form a closed circulation. The Oyashio waters move south along the Asiatic Coast to meet the Kuroshio waters advancing from the south. The two waters turn eastward mingling, and cross the ocean in a broad movement in which there is a south-north gradient of properties. The northern side of this current, consisting chiefly of Oyashio water, circulates around the Gulf of Alaska and is largely dissipated into the Bering Sea where it rejoins the Oyashio.

Later summer data confirms the principal features of this circulation pattern. It was originally imagined that the system might be accelerated in winter and continue by virtue of momentum through the summer. However, the winter data show that the circulation is not significantly different in this season.

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DISCUSSION

K. HIDAKA: Salinity minima at the surface are hard to explain when we seek the origin of deep waters.

F. NEAVE: Low surface salinity is widespread until you get down below latitude 36° N.

CURRENTS AND WATER STRUCTURE IN QUEEN CHARLOTTE SOUND, BRITISH COLUMBIA

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Data are available from recent oceanographic surveys of the oceanic and coastal waters of the northeast Pacific, and particularly of these waters in the Gulf of Alaska. One feature of the area is an excess of precipitation over evaporation. This excess, together with an accumulation of the fresh water in the surface layers, is evidenced in the salinity structure which is observed over the entire gulf region. At the surface, salinities are everywhere less than 33.0°/$_{oo}$ generally decreasing toward the coasts.

There is an upper zone of relatively homogeneous low salinity water, less than 33.0°/$_{oo}$ extending to about 100 meters depth. Below this, and to a depth of about 170 meters, is a well-defined halocline in which the salinity increases to 33.8°/$_{oo}$. This halocline is a boundary separating the upper zone from a deep zone where the salinity is greater than 33.8°/$_{oo}$ and increases with depth.

This structure is observed during all seasons.

The excess of precipitation is greatest near

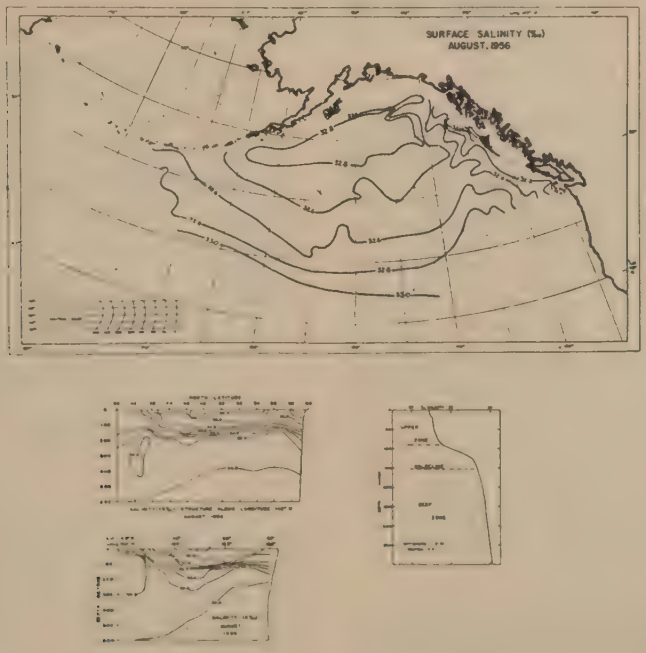

Fig. 1.—Surface salinity, salinity in longitudinal section and structure in the Gulf of Alaska region.

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the coasts where, in addition, large volumes of fresh water from land drainage enter the sea. The seaward entry of this land drainage very often occurs only after passage through extensive coastal seaways in which conditions of extreme tidal turbulence occur. By the time the sea is reached, the outflowing water has attained a salinity very near that of surface zone water in the ocean.

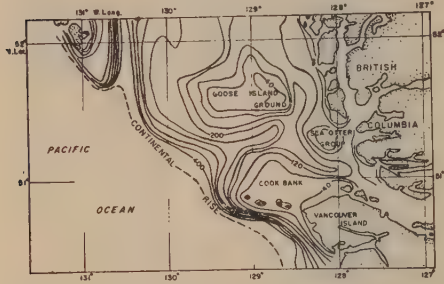

Fig. 2.—Chart of Queen Charlotte Sound.

A characteristic of the coast along the eastern periphery of the Gulf of Alaska is that there are many such seaways; a representative example is that of Queen Charlotte Sound. The principal features of the sound are shown in Fig.2. The Pacific Ocean and the continental rise lie to the west, the mainland of British Columbia to the east. Extensive shallow areas and deep trenches extending from seaward are marked features. One of the principal trenches is that which lies between Cook Bank and the Goose Island Ground and extends into the seaway between Vancouver Island and the mainland. As can be seen, there are numerous coastal indentations and seaways close to this principal channel.

The distribution of surface salinity during the summer (June, 1955) is indicated in Fig.3. The fact that the greatest volume of fresh water reaches the sound in the vicinity of the principal channel is indicated by the tongue of relatively low salinity water extending seaward from this region.

The summer distribution of salinity in a vertical section through the principal channel is shown in Fig.3. Between the surface and 50 meters depth, relatively low salinity water less than 32.5°/$_{oo}$ dominated. The effect of the freshwater outflow from adjacent channels is reflected in the lower salinity values observed at the surface in mid-section. Below about 50 meters depth, the salinity is everywhere greater than 32.8°/$_{oo}$ and increases with depth to at least 33. 6°/$_{oo}$. As we saw, these are the same salinities as observed in the oceanic halocline. It is evident that oceanic waters have intruded well into the inner reach of the seaway, as there is no other source of water in the coastal region.

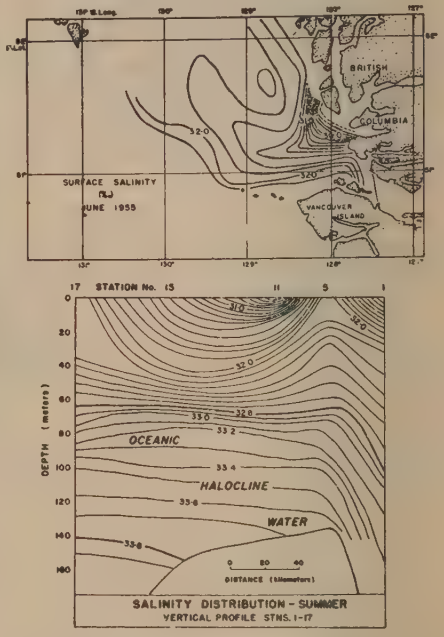

Fig. 3.—(a) Surface salinity summer.
(b) Salinity in a section through the principal channel.

It is interesting to note that the range of salinities and the structure at seaward end of this section are very nearly the same as those observed farther seaward in the Gulf of Alaska. It is evident that the oceanic structure is attained within the coastal regions.

At positions in the vicinity of the principal channel, hourly Ekman current observations

were made for periods up to 50 hours. The data were analysed to obtain the net movement at each depth at each position.

These net movements as calculated for two of the more seaward stations indicated a persistent movement of the deep and bottom water into the sound and toward the inner reach of the principal channel.

The salinity identifies the deep water as coming from the region of the oceanic halocline. The current data show that it is persistently intruding the coastal seaway being entrained and dissipated in the near surface waters. The shoreward movement of deep waters is consequent on the upward transport of this water into the surface seaward-moving layers.

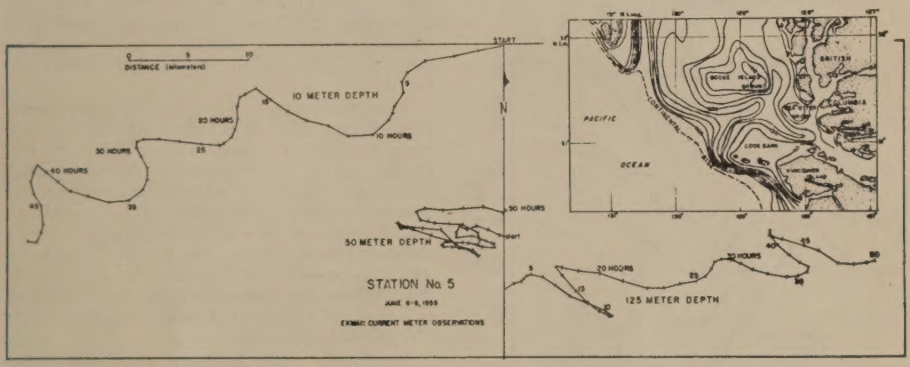

Fig. 4.—Current observations at 10, 50, and 125 meter depths at Station 5.

Data obtained in the shoreward end of the channel during a 50-hour period are indicated in Fig. 4. At the 10-meter depth a seaward movement occurred which, during a tidal day was about 20 kilometers. At the 50-meter depth it can be seen that little net movement occurred. At the 125-meter depth (the deepest depth of observation at this position), a large net movement again occurred; it was, however, directed shoreward and about 15 kilometers per day.

The vertical distribution of net movement at this position is summarized in Fig. 5. Above 50 meters, a net seaward movement occurred with a maximum rate of over 20 cm/sec at the surface, decreasing steadily to nearly zero at 50 meters. Below 50 meters, a movement into the seaway occurred which, between 75 and 125 meters, averaged about 15 cm/sec.

In the deep water, therefore, the inflow is continuous from seaward well into the inner reach of the principal channel. The contrary directions of the surface and deep water indicate the existence of considerable shear with consequent mixing occurring at all depths.

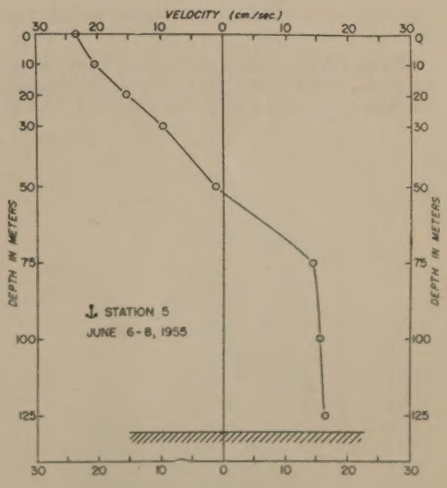

Fig. 5.—Vertical profile of net movement at Station 5.

It is known that the maximum land drainage into this region occurs during the summer, a minimum in early spring; and while no quantitative assessment has been made of the seasonal nature of the discharge through the seaway, it is considered that sufficient surface outflow occurs to allow the entrainment process, and hence the deep inflow, to persist during all seasons. This leads to a consideration of the source water comprising the deep inflow.

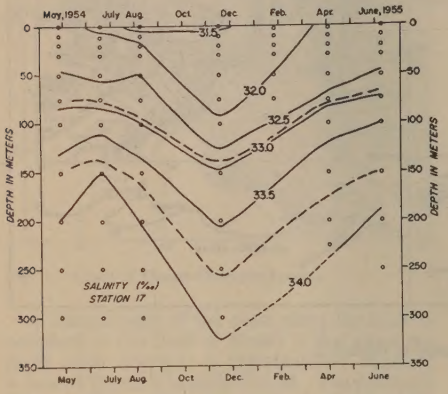

Fig. 6.—Seasonal variation of the depth of the halocline.

During the prevalence of southerly winds along the coast, usually strongest from autumn through the winter to early spring, the oceanic halocline is depressed below the level of the continental shelf. The alternate condition is associated with light or northwest winds, and maximum run-off when the oceanic halocline rises along the coast. These conditions occur most frequently during the summer.

This depth variation of the halocline leads to marked changes in the salinity of the deep inflow which is in turn reflected in changes of the salinity of the water in the entire system. In December, 1954, after a prolonged period of southerly winds, the salinity of sound water was about $1^{o}/_{oo}$ less at all depths than during the summer.

These variations in the salinity of source water then of deep inflow have a profound effect on the characteristics of coastal water. This is further emphasized by the fact that the period at which the salinity of sound water is a maximum occurred at a time of maximum run-off. Evidently the character of the water is dominated more by the deep inflow than by the amount of run-off. The same conclusion regarding the effect of the deep inflow has been expressed by Waldichuk in a study of the Strait of Georgia system—some 200 miles farther south.

Finally, in these seaways fresh and ocean waters are mixed to ocean structure within the limits of the coastal seaway. The deep inflow provides a mechanism through which oceanic conditions are reflected in inshore waters such as these. A consideration of all the data suggests that complete renewal of the deep and bottom water could occur in a period as short as two weeks. This may be of considerable significance in this important fishing area.